

# Morphological and molecular evidence support the intertidal barnacle *Octomeris intermedia* Nilsson-Cantell, 1921 (Thoracica, Chthamalidae) as a valid species in Indo-Pacific waters

Benny K.K. Chan<sup>1</sup>, Yao Feng Tsao<sup>1</sup>, Monthon Ganmanee<sup>2</sup>

**1** Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan **2** Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Chalongkrung Road, Ladkrabang, Bangkok 10520, Thailand

Corresponding author: Monthon Ganmanee ([monthon.ga@kmitl.ac.th](mailto:monthon.ga@kmitl.ac.th))

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## Abstract

*Octomeris* is a chthamalid intertidal barnacle with eight shell plates. There are currently two species of such barnacles: *O. brunnea* Darwin, 1854 (type locality in the Philippines), common in the Indo-Pacific region, and *O. angulosa* Sowerby, 1825, only recorded in South Africa. *Octomeris intermedia* Nilsson-Cantell, 1921, identified from the Mergui Archipelago in Myanmar, was considered to be conspecific with *O. brunnea* by Hiro (1939) based on samples collected in Taiwan. The morphological differences in shell and opercular plates between *O. brunnea* and *O. intermedia* are believed to be intra-specific variations due to different degrees of shell erosion. In the present study, the genetic and morphological differentiations of *Octomeris* in the Indo-Pacific region were examined. This study found two molecular clades (with inter-specific differences) based on the divergence in the COI genes, and the species also have distinct geographical distributions. The *Octomeris brunnea* clade covers samples collected from the Philippines and Taiwan waters and the other clade, which we argue is *O. intermedia*, is distributed in Phuket and Krabi, Thailand and Langkawi, Malaysia. Phuket and Krabi are located approximately 300 km south of the Mergui Archipelago, the type locality of *O. intermedia*. The morphology of samples collected from Thailand fits the type description of *O. intermedia* in Nilsson-Cantell (1921). Our study concludes that *O. intermedia* is a valid species based on morphological and molecular evidence.

## Keywords

Barnacles, biogeography, molecular taxonomy

## Introduction

*Octomeris* is a chthamalid intertidal barnacle with eight shell plates, in contrast to most of chthamalids which have four or six shell plates. In the early 19<sup>th</sup> century, studies on the biology and ecology of *Octomeris* were very rare because this species inhabits shaded habitats and its presence was often overlooked (Nilsson-Cantell 1938). A recent molecular phylogenetic analysis of the family Chthamalidae included two species of *Octomeris* (Pérez-Losada et al. 2012) and considered *Octomeris* as paraphyletic; molecular evidence does not support the hypothesis that plate number decreased from eight plates to six, then four in the chthamalid evolution (Pérez-Losada et al. 2012).

In the Indo-Pacific region, *Octomeris* was considered to be composed of four species: *O. brunnea*, *O. angulosa*, *O. sulcata*, and *O. intermedia*. *Octomeris sulcata* has a strongly fused scutum and tergum, and Poltarukha (1996) relocated *O. sulcata* to the monotypic genus *Pseudoctomeris*. Chan et al. (2017) repositioned *Pseudoctomeris* from Chthamalidae to Pachylasmatidae based on multiple marker molecular analyses, leaving three species in *Octomeris*. *Octomeris brunnea* was described by Darwin (1854) from the Philippine archipelago; it has a brown colored depressed shell and longitudinal furrows on its surface. The tergal and scutal margins of the opercular plates are straight. *Octomeris angulosa* was described by Sowerby (1825) from the Cape of Good Hope in South Africa as having a dirty white strong conical shell and coarsely crenated shell plates (see re-description in Darwin 1854). *Octomeris angulosa* is common in wave-exposed shores in South African waters and often interacts with *Tetraclita serrata* (Boland 1997). *Octomeris intermedia* was described by Nilsson-Cantell (1921) from Java (note the erratum on the type locality (South Atlantic Ocean in Nilsson-Cantell, 1921) stated in Nilsson-Cantell (1937); Fig. 1), having a depressed shell and sinuous tergal and scutal margin. Nilsson-Cantell (1938) further recorded *O. intermedia* in the Mergui Archipelago in the Malay Peninsula (Fig. 1). Hiro (1939), however, collected a different size range of *O. brunnea* in Taiwan and observed that there is great variation in the shape of scutum and tergum at different ages and with different degrees of erosion. Highly eroded large individuals have a smooth flattened shell and a sinuous tergal and scutal margin, which resemble the morphology of *O. intermedia*. Juvenile and un-eroded specimens represent the morphology of *O. brunnea*, which has longitudinally furrowed shells and straight scutum and tergum junction. Hiro (1939) concluded that *O. intermedia* and *O. brunnea* are conspecific. The conclusion of Hiro (1939) was further supported by Pope (1965), who examined *O. brunnea* in Australian waters and suggested that *O. intermedia* is an older specimen of *O. brunnea*. However, Hiro (1939) did not include *O. intermedia* in the geographical range suggested by Nilsson-Cantell (1921, 1938) (Java and the Mergui Archipelago), nor did the former compare the latter's samples of *O. brunnea* collected from Taiwan. To further test the conclusion by Hiro (1939), a combined morphological and molecular approach, known as integrative taxonomy (Dayrat 2005), is needed to compare *O. intermedia* collected from Java, Mergui Archipelago, and their adjacent waters with *O. brunnea* and ascertain the taxonomic status of these two species. In the present study, we collected *O. intermedia*

from Phuket and Krabi, Thailand (300 km south of the Mergui Archipelago) and Langkawi, Malaysia, and *O. brunnea* from Taiwan and the Philippines; these samples cover different sizes and degrees of erosion. The mitochondrial cytochrome C oxidase subunit I (COI) and 12S rRNA genes were used as genetic markers to test the hypothesis that *O. intermedia* is an eroded form of *O. brunnea* in the Indo-Pacific region.

## Materials and methods

### Study sites and sample collections

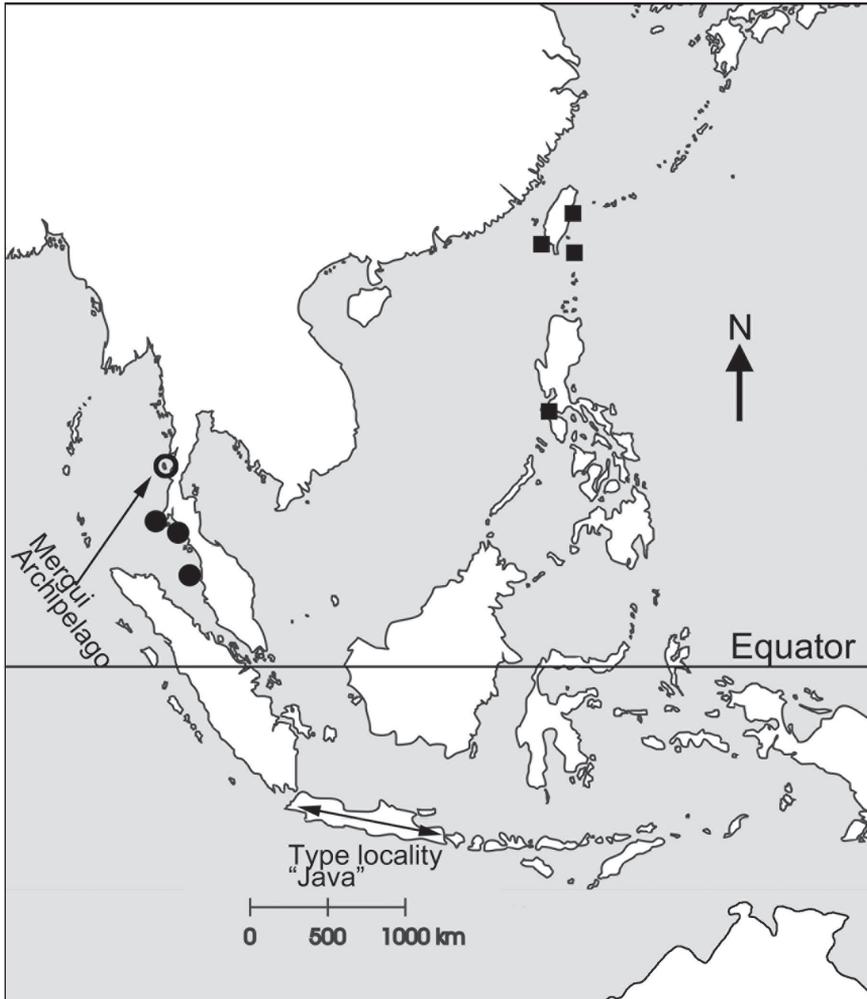
Samples of *Octomeris intermedia* were collected from Hey (or Coral) Island, Phuket (7°44'47" N; 98°22'44 E) and Ao Nang Beach, Krabi (8°02'08" N; 98°48'57 E), Thailand and Langkawi, Malaysia (Fig. 1). *Octomeris* in Thailand inhabits shaded rocks in the high intertidal zone, especially on vertical rock surfaces or shaded overhang surfaces in intertidal sea caves (Figs 1, 2). They can reach a percentage cover of 100% in some of the shaded rocks (Fig. 2C). Samples of *O. brunnea* (Fig. 2G) were collected in Kenting, Green Island, and Lanyu Island in Taiwan and Puerto Galera in the Philippines (Fig. 1). *Octomeris brunnea* was found on shaded rock surfaces and, occasionally, sun-exposed rocks. The abundance of *O. brunnea* was not high, with only a few individuals colonizing a shaded area. Representative specimens were deposited in the Biodiversity Research Museum, Academia Sinica (ASIZCR) and Collections in the first author's laboratory (CEL).

### Morphological analysis

Before dissection, the shape of the junction line of scutum and tergum was recorded for all specimens with different size ranges. The morphological characters of shell parts (wall plates, scutum, and tergum) and somatic bodies (six pairs of cirri, penis, and trophi) were examined. The shells and opercular plates (scuta and terga) were immersed in 20% bleach for ca. 20 minutes to completely dissolve organic tissues, rinsed by purified water for 5 minutes, and air-dried. The cirri, penis, and trophi were dissected, mounted on glass slides, and observed under a light microscope (Olympus BX60, Japan). The morphology of the setae was described following the terminology of Chan et al. (2008).

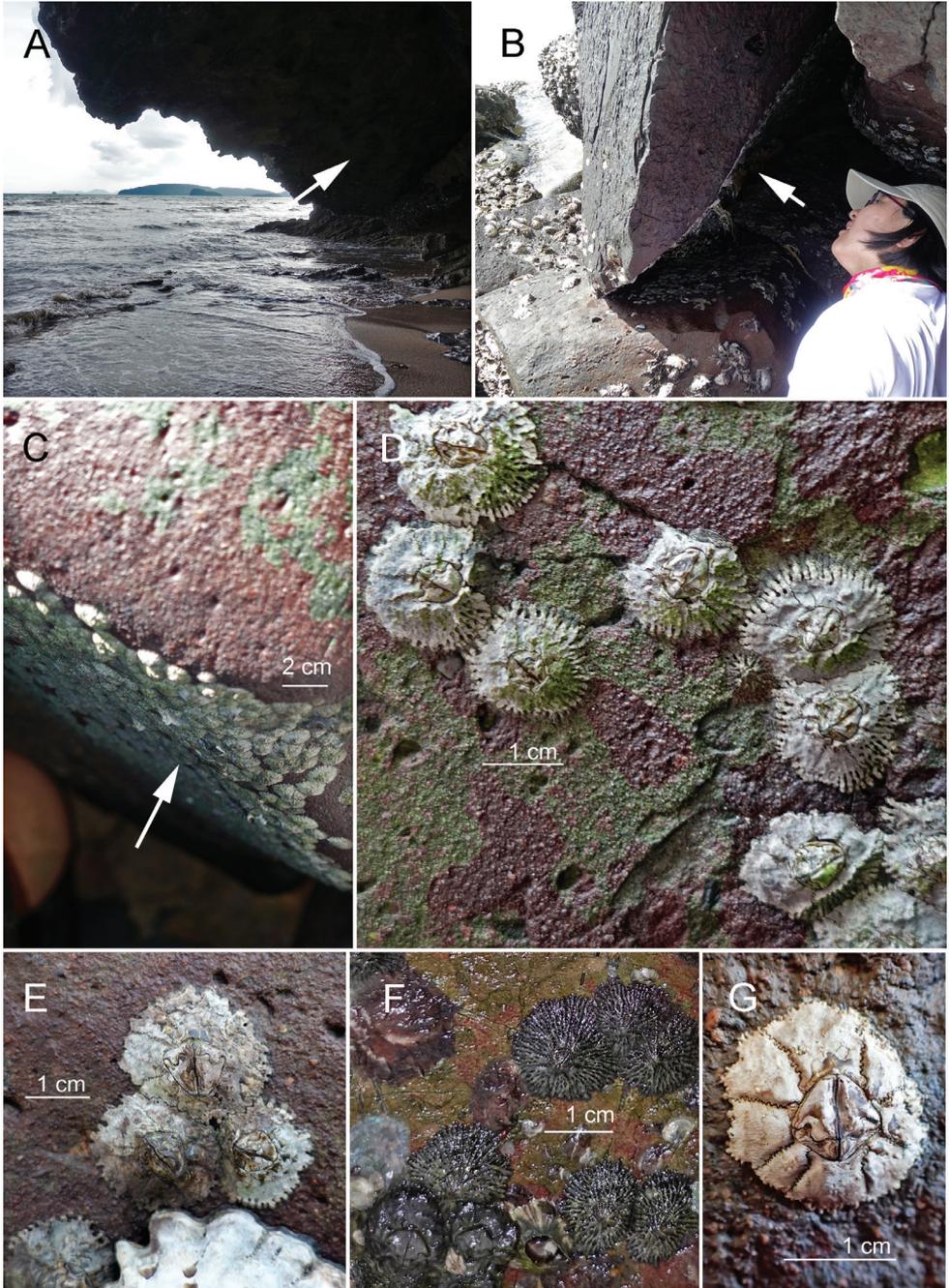
### DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from the muscle tissue using Qiagen DNeasy® Blood & Tissue Kits (Qiagen, California, USA) according to the manufacturer's instructions. Partial sequences of mitochondrial DNA markers, COI, and 12S rRNA were amplified to reconstruct phylogenetic relationships. The primers used to amplify the sequences in the polymerase chain reaction (PCR) were LCO1490 and



**Figure 1.** Sampling locations for *Octomeris intermedia* (black circles) and *Octomeris brunnea* (black squares). Open circle indicates the sampling location of *O. intermedia* in the Mergui Archipelago stated in Nilsson-Cantell (1938).

HC02198 for COI (Folmer et al. 1994) and 12S-F1 and 12S-R1 for 12S rRNA (Mokady et al. 1994). The PCR solution contained approximately 100–200 ng of template DNA, 0.4  $\mu$ L each of 10  $\mu$ M primer, 4  $\mu$ L of Fast-Run™ *Taq* Master Mix with Dye (Protech Technology Enterprise, Taipei, Taiwan), and ddH<sub>2</sub>O to the final volume of 20  $\mu$ L. PCR reactions were conducted in a DNA Engine Thermal Cycler (Bio-Rad, Richmond, California, USA). The thermal cycle began with an initial denaturation at 94°C for 4 min, then 35 cycles of denaturation at 94°C for 1 min, annealing at 49–51°C for 30 sec, and an extension at 72°C for 1 min (COI) and 30 sec (12S rRNA). The final extension step was at 72°C for 10 min. PCR products were checked by electrophoresis on 1.5 % agarose gel in 1  $\times$  TAE buffer. DNA purification and Sanger DNA sequencing were performed by Genomics BioSci & Tech Ltd.



**Figure 2.** **A** Krabi, Thailand, showing *O. intermedia* at the overhang of an intertidal cave (indicated by white arrow). **B** Hey or Coral Island, Phuket, showing that *O. intermedia* occur on shaded rocks. **C** *O. intermedia* can occupy up to 100% cover under a shaded rock in Thailand. **D** *Octomeris intermedia*, showing partially eroded samples, with longitudinal furrows at the base of the shell plates. **E** *O. intermedia*, eroded samples, showing shell with a smooth surface. **F** *O. intermedia*, uneroded samples, showing longitudinal furrows on the surface. **G** *O. brunnea* on Lanyu, Taiwan. Shell is more conical than *O. intermedia*. Eroded sample with smooth shell plates.

(New Taipei City, Taiwan). The sequences were assembled and edited in Geneious 7.0.6 (<https://www.geneious.com>).

## Phylogeny reconstruction and genetic distances

The phylogenetic trees were reconstructed from COI and 12S rRNA sequences using neighbor-joining (NJ), Bayesian inferences (BI), and maximum likelihood (ML) conducted in MEGA X 10.0.5, MrBayes 3.2.6, and W-IQ-TREE, respectively (Kumar et al. 2018; Nguyen et al. 2015; Ronquist and Huelsenbeck 2003; Trifinopoulos et al. 2016). Selected sequences of Chthamalidae downloaded from GenBank were included in the analysis, and the *Catomerus polymerus* sequence was used as the outgroup (Chan et al. 2018; Chen et al. 2019; Fisher et al. 2004; Pérez-Losada et al. 2004; Pérez-Losada et al. 2012; Wares 2013; Wares et al. 2009) (Table 1).

All the sequences were aligned with ClustalW implemented in Geneious 7.0.6 (<https://www.geneious.com>). Neighbor-joining trees were generated on the analysis of Kimura 2-parameter (K2P) distances with bootstrap values estimated from 1,000 pseudoreplicates for two markers, separately (Felsenstein 1985; Kimura 1980; Saitou and Nei 1987). Bayesian inferences were conducted with  $2 \times 10^6$  generations of the MCMC chain. Trees were saved every 1000 generations, and the first 500,000 trees (25%) were discarded as burn-in. Maximum likelihood was conducted with 1,000 bootstrap replicates for a Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT) and ultrafast bootstrap approximation (UFB) (Guindon et al. 2010; Hoang et al. 2017). GTR+F+I+G4 and TVM+F+G4 were selected as the best-fit model under the Bayesian information criterion for COI and 12S rRNA, respectively (Kalyaanamoorthy et al. 2017). Genetic distances (K2P) between and within species were calculated by MEGA X 10.0.5 (Kumar et al. 2018).

## Results

### Systematics

#### Family Chthamalidae

#### Subfamily Notochthamalinae

#### Genus *Octomeris* Sowerby 1825

#### *Octomeris brunnea* Darwin, 1854

Figures 2G, 3–9, 15D–F, 16D–F

*Octomeris brunnea* Darwin, 1854: 484, pl 20, figs 3a, b; Weltner 1897: 274; Gruvel 1905: 197, fig. 217; Nilsson-Cantell 1921: 299, figs 58, 59, pl 3, fig. 7; –1931: 108; –1932, 14; Hiro 1939: 252, figs 3–4, 6a, b; Utinomi 1949: 25; –1958: 307; Endean, Kenny and Stephenson 1956: 122, 127, tab. 1; Endean, Stephenson and

**Table 1.** Specimen information and GenBank accession numbers for DNA sequences used in this study.

Species	Specimen voucher	Locality	COI	12S	Reference
<i>Octomeris brunnea</i>	KT_131_02	Haikou, Pingtung, Taiwan	MN928617	MN928665	This study
	LAN_178_01	Lanyu, Taiwan	MN928618	MN928668	
	LAN_178_02		MN928619	MN928669	
	Octm_b_02	Haikou, Pingtung, Taiwan	MN928620	MN928670	
	Octm_b_03		MN928621	MN928671	
	Octm_G05_01	Puerto Galera, Mindoro, Philippines	MN928622	MN928672	
	Octm_G05_03		MN928623	MN928673	
	Octm_G05_04		MN928624	MN928674	
	Octm_G05_05		MN928625	MN928675	
	Octm_G23_01		MN928626	MN928676	
	Octm_GI_01	Green Island, Taiwan	–	MN928677	
	Octm_GI_02		–	MN928678	
	Octm_GI_03		–	MN928679	
	Octm_sp_01	Shihtiping, Hualien, Taiwan	MN928627	MN928684	
	Octm_TW_02	Haikou, Pingtung, Taiwan	MN928628	MN928685	
	Octm_TW_05		MN928629	MN928686	
	Octm_TW_06		MN928630	MN928687	
	Octm_TW_07		MN928631	MN928688	
	Octm_TW_08		MN928632	MN928689	
	Octm_TW_09		MN928633	MN928690	
Octm_TW_10	MN928634		MN928691		
Octm_TW_11	MN928635		MN928692		
<i>Octomeris intermedia</i>	CEL_Thai_243_01		Hey Island, Phuket, Thailand	MN928636	MN928655
	CEL_Thai_243_02			MN928637	MN928656
	CEL_Thai_243_03	MN928638		MN928657	
	CEL_Thai_243_04	MN928639		MN928658	
	CEL_Thai_243_05	MN928640		MN928659	
	CEL_Thai_243_06	MN928641		MN928660	
	CEL_Thai_243_07	MN928642		MN928661	
	CEL_Thai_243_08	MN928643		MN928662	
	CEL_Thai_243_09	MN928644		MN928663	
	CEL_Thai_243_10	MN928645		MN928664	
	Octm_MA_01	Langkawi, Malaysia	MN928646	MN928680	
	Octm_MA_02		MN928647	MN928681	
	Octm_MA_03		MN928648	MN928682	
	Octm_MA_04		MN928649	MN928683	
	Thai_359_03	Ao Nang Beach, Krabi, Thailand	MN928650	MN928693	
	Thai_359_05		MN928651	MN928694	
Thai_359_06	MN928652		MN928695		
<i>Octomeris intermedia</i> *		Phuket, Thailand	AY430812	–	Fisher et al. 2004
			–	JX083940	Pérez-Losada et al. 2012
<i>Catomeris polymerus</i>		Coledale Beach, Wollogong, Australia	MH791045	MH791045	Chan et al. 2018
<i>Chamaesipho columna</i>		Devenport, New Zealand	JX083866	JX083937	Pérez-Losada et al. 2012
<i>Chamaesipho tasmanica</i>		Tasmania, Australia	JX083867	–	
			–	AY520681	Pérez-Losada et al. 2004
<i>Chthamalus challengeri</i>		Jiangsu, China	KY865097	KY865097	Chen et al. 2019
<i>Hexechamaesipho pilsbryi</i> 2	LAN_173_01	Lanyu, Taiwan	MN928653	MN928666	This study
<i>Hexechamaesipho pilsbryi</i> 1	LAN_173_03		MN928654	MN928667	
<i>Microeuraphia rhizophorae</i>		Panama	FJ845866	–	Wares et al. 2009
<i>Microeuraphia rhizophorae</i>		Brazil	–	JX083950	Pérez-Losada et al. 2012
<i>Nesochthamalus intertextus</i>		Japan	JX083869	JX083942	
<i>Notochthamalus scabrosus</i>		Arica, Chile	NC_022716	NC_022716	Wares 2013
<i>Octomeris angulosa</i>		Cape Town, South Africa	AY428049	–	Fisher et al. 2004
<i>Octomeris angulosa</i>		Sydney, Australia	–	JX083939	Pérez-Losada et al. 2012
<i>Pseudoctomeris sulcata</i>		Japan	JX083865	JX083936	

\* The sequences of *O. intermedia* from Fisher et al. (2004) and Pérez-Losada et al. (2012) were designated as *O. brunnea* in their studies.

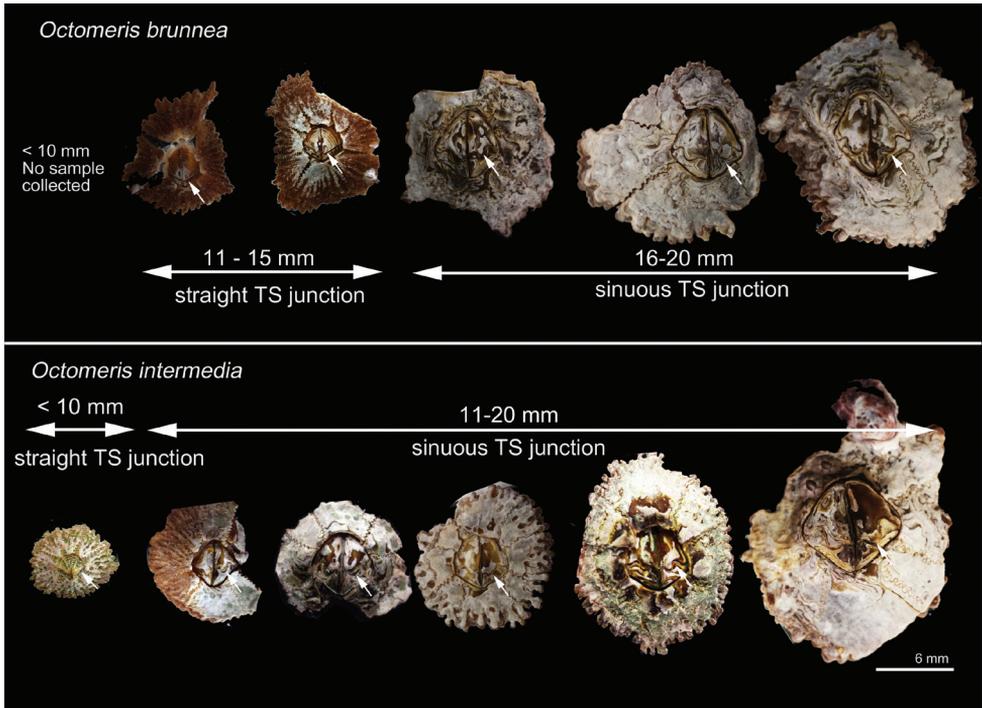
Kenny 1956: 332, 336, app II; Pope 1965: 20, figs 1c, 2b, pl 1: figs 3, 6; Newman and Ross 1976: 40; Poltarukha 1996: 992; Liu and Ren 2007: 283, fig. 123; Chan et al. 2009: 153: figs 128–130; Jones 2012: tabs 1, 2.

**Materials examined.** ASIZCR-000431. Intertidal rocks at General Rock, Green Island, Taiwan (22°40.35'N, 121°29.45'E, 16 August 2019, 1 specimen). CEL-Octm\_GL\_01. Intertidal rocks at General Rock, Green Island, Taiwan (22°40.35'N, 121°29.45'E, 16 August 2019, 5 specimens). CEL-KT-131. Intertidal rocks at Hai Kou, Kenting, Taiwan (22°06.06'N, 120°42.56'E, 4 Dec 2007, 7 specimens). CEL-LAN-178. Intertidal rocks at southern Lanyu, Lanyu, Taiwan (22°00.82'N, 121°33.94E, 19 June 2019, 2 specimens). CEL-Octm\_sp\_01. Intertidal rocks at Shi-Ti-Ping, Hualien, Taiwan (23°28.56'N, 121°30.41E, 13 May 2009, 1 specimen). CEL-Octm-G05. Intertidal rocks at Puerto Galera, Philippines (02 June 2009, 20 specimens). CEL-Octm-G23. Intertidal rocks at Varadaro Point, Puerto Galera, Philippines (02 June 2009, 1 specimen).

**Diagnosis.** Shell eight plated, conically depressed. Shell brown, surface with longitudinal furrows and tergo-scutal junction straight in young and uneroded specimens. Shell gray, surface smooth and tergo-scutal junction sinuous in old and eroded specimens. Maxillule with deep notch at upper 1/3 of cutting edge, lower 1/3 strongly protruded, cutting edge clearly divided into upper, middle, and lower region by the clear notch and protrusion of lower margin.

**Description.** Shell 8 plated, composed of piece of rostrum (R), carina (C), paired rostro-lateral (RL), carino-lateral (CL) and lateral (L) (Figs 2G, 4A, B). Shell conically depressed. Shell brown, surface with longitudinal furrows and tergo-scutal junction straight in young and uneroded specimens (Figs 3, 4A). Shell grey, smooth, tergo-scutal junction sinuous in old and eroded specimens (Figs 3, 4B). Sutures of shell plates serrated (Figs 2G, 4A, B). In young and uneroded specimens, scutum triangular, outer surface with horizontal growth lines (Fig. 4A). Scutum inner surface brown, tergal and occludent margins straight in young specimens, basal margin slightly convex (Fig. 4A). Tergal margin straight, with conspicuous articular ridge. Adductor muscle scar shallow (Fig. 4A). In older and eroded specimens, tergal margin of scutum strongly sinuous, adductor muscle scar deep (Fig. 4B). Tergum with basal margin strongly bended in an angle, scutal margin straight with deep articular ridge in young specimens, depressor muscle distinct, muscle crests prominent and extended slightly out of the carinal margin of tergum (Fig. 4A). In older and eroded specimens, the scutal margin strongly concaved (Fig. 4B).

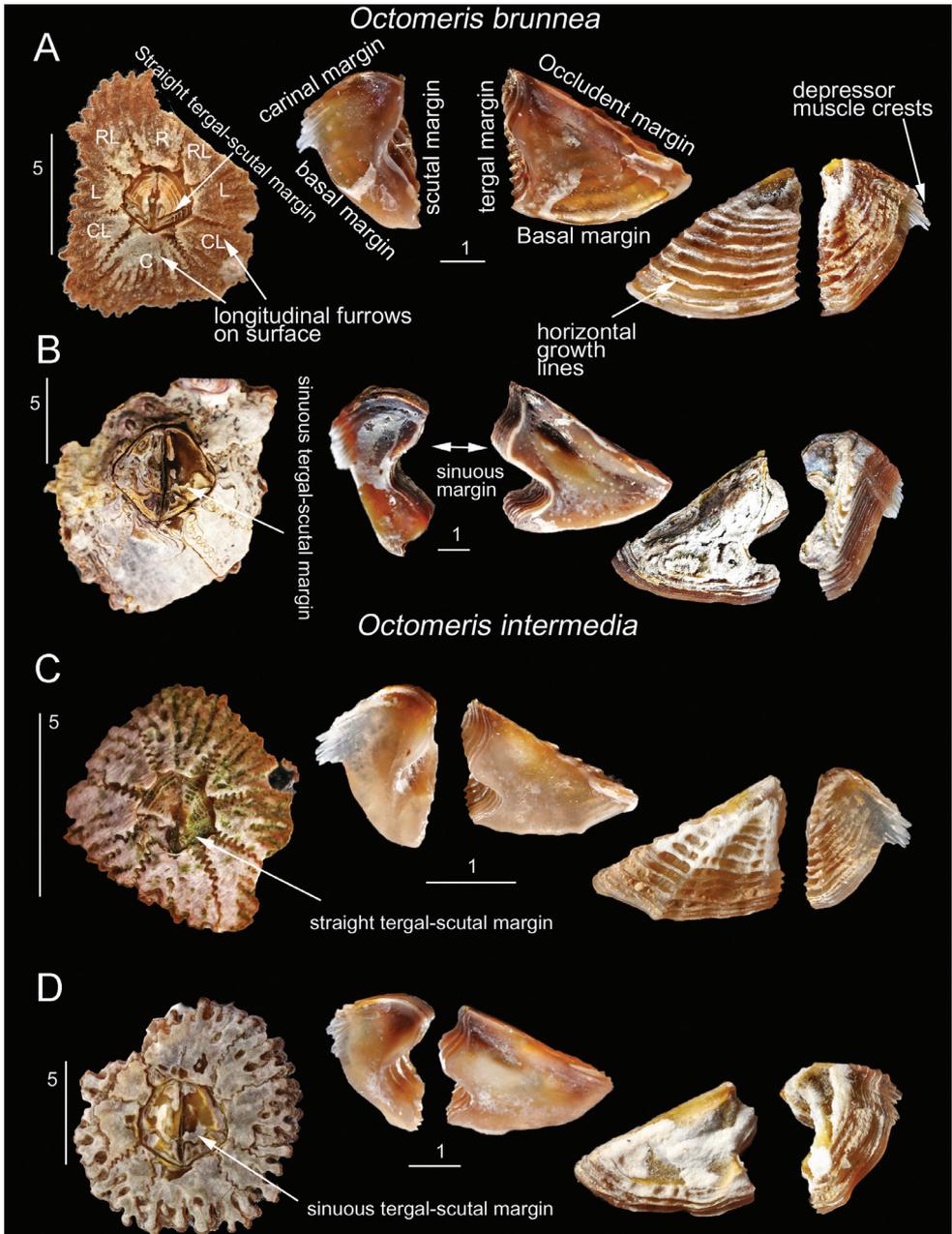
Cirrus I rami unequal (Fig. 5A). Posterior ramus short, six-segmented. Anterior ramus seven-segmented. All segments height greater than width (Fig. 5A). Bidentate serrate setae and simple setae present. Bidentate serrate setae appear up to seven segments in anterior ramus and present up to first three distal segment in posterior ramus (Fig. 5B–D). Cirrus II, posterior ramus seven-segmented, anterior ramus eight-segmented. Bidentate serrate setae present up to seven segments in anterior ramus and up to first four distal segments in posterior ramus (Fig. 5E–H). Cirri III to VI similar in



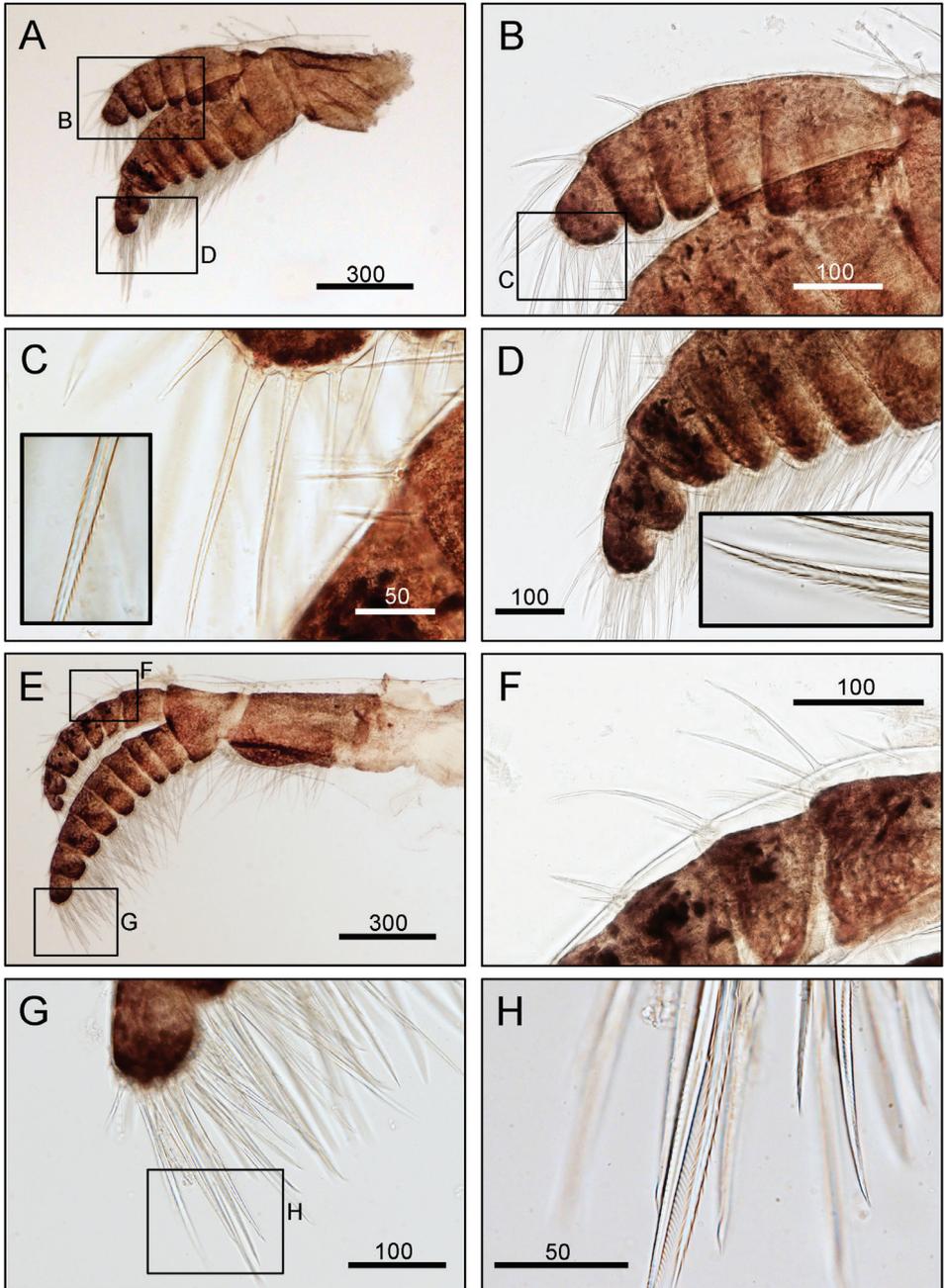
**Figure 3.** Shape of the tergo-scutal junction (TS junction, indicated by arrows) of *Octomeris brunnea* (CEL-KT-131, Hai Kou, Taiwan) and *O. intermedia* (CEL-Thai-359, Krabi, Thailand). All specimens share the same scale bar.

morphology, long and slender (Figs 6, 7). Cirrus III, posterior and anterior rami 12 segmented (Fig. 6A–D). Cirri IV and V, posterior and anterior rami 15-segmented (Figs 6E–H, 7A–C). Dorsal surface of cirri IV–VI has small spines (Figs 6G, 7F). Cirrus VI with 16 segmented rami (Fig. 7D–E). Intermediate segments of cirri III and VI with three pairs of long and one pair of short simple setae (Figs 6B, F, 7B, E). Distal segments of cirrus III bear two pairs of long and one pair of short setae (Figs 6C, D, H, 7C). Caudal appendage absent. Penis short, tip with a few simple setae (Fig. 7G, H).

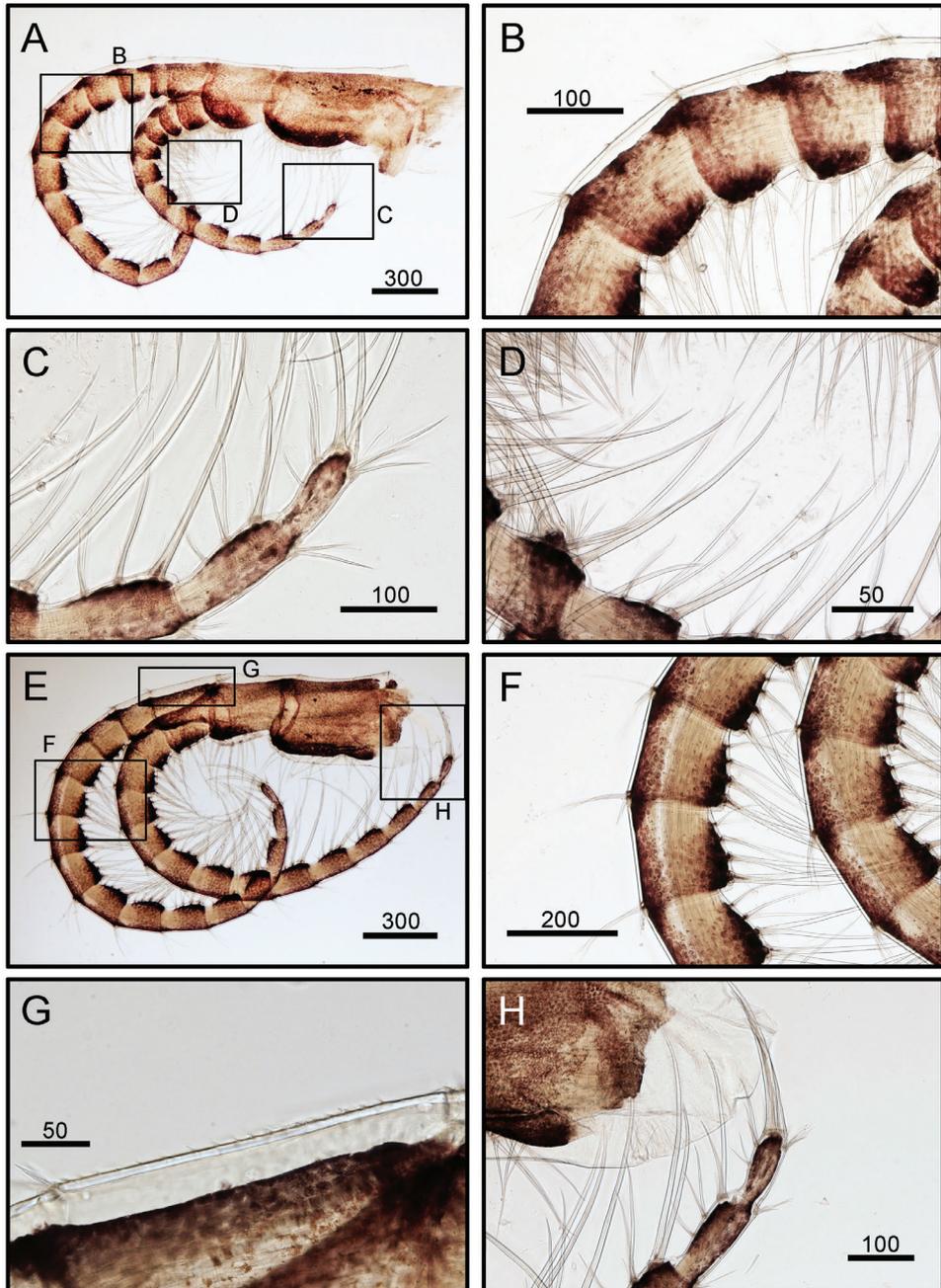
Maxilla subtriangular, distal lobe prominent and proximal lobe flat, shallow notch present in inner margin between the two lobes (Fig. 8A), inner and outer margin with serrulate setae (Fig. 8B–D). Maxillule with a deep notch on upper 1/3 and lower 1/3 of cutting edge. Cutting edge obviously divided into three distinct portions. Cutting edge above upper notch with two large and a few setae (length of setae ranges from 80–100  $\mu\text{m}$ ); middle portion of cutting edge has six setae; 1/3 of lower portion of cutting edge has eight short setae (Fig. 8E–H). Mandibles with three teeth, cutting edge of first tooth smooth, second tooth with one or two spines, third tooth with a few spines on cutting edge (Fig. 9A–D). Mandibular palp elongated, with serrulate setae on outer margin (Fig. 9E, F). Cutting margin of labrum concave, with small fine teeth (Fig. 9G, H).



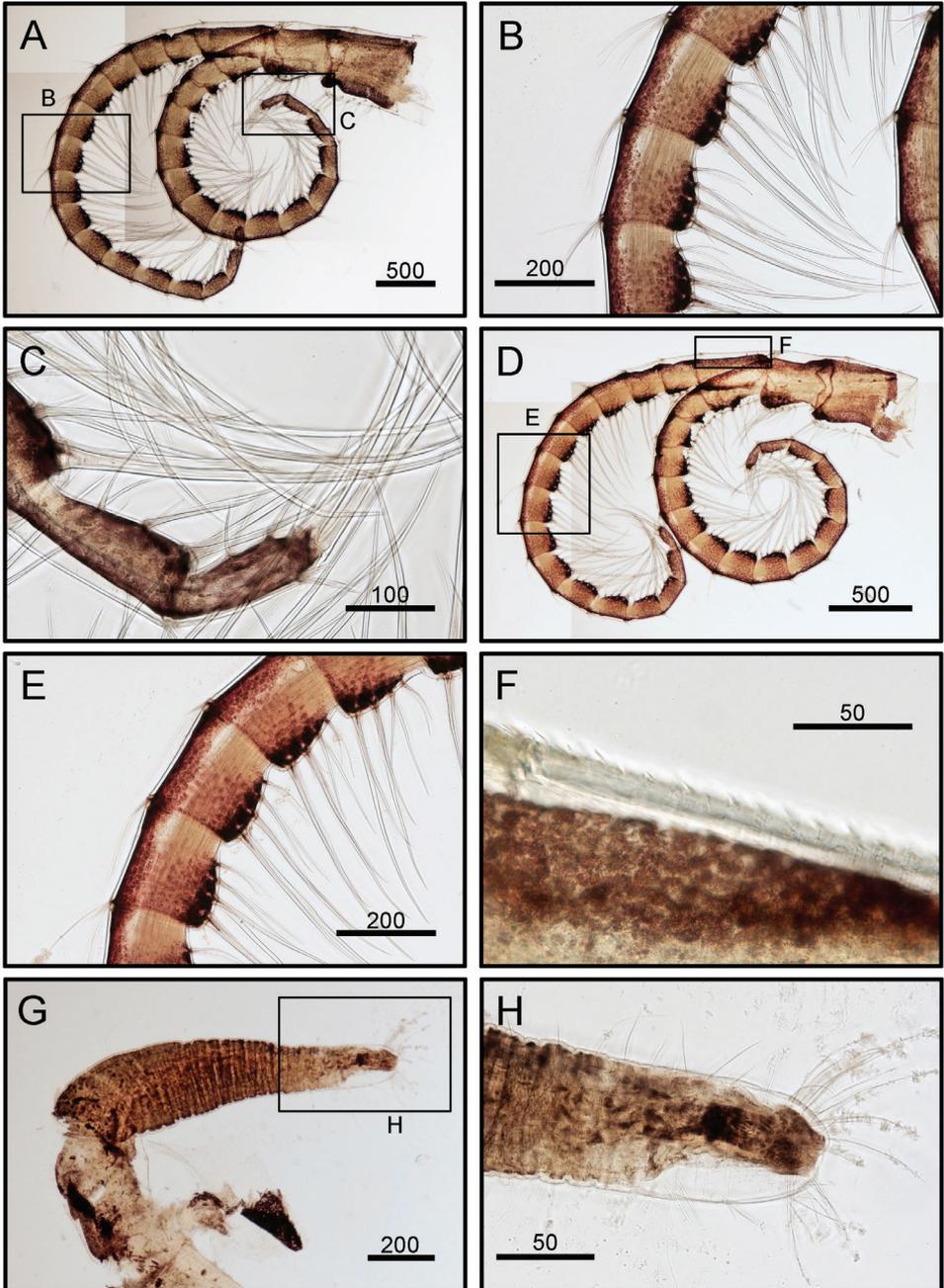
**Figure 4.** *Octomeris brunnea* (CEL-KT-131, Hai Kou, Taiwan) and *O. intermedia* (CEL-Thai-359, Krabi, Thailand). **A** *Octomeris brunnea*, young specimens showing the straight tergo-scutal junction and the inner and outer sides of left scutum and tergum. **B** *O. brunnea*, older eroded specimen, showing the sinuous tergo-scutal junction and inner and outer sides of left scutum and tergum. **C** *O. intermedia*. Very small individual (shell length < 10 mm) showing the straight tergo-scutal margin and inner and outer sides of scutum and tergum. Note only very small individuals of *O. intermedia* have straight tergo-scutal margin. **D** *O. intermedia*. Larger specimens, showing the sinuous tergo-scutal margin and inner and outer surfaces of scutum and tergum. Scale bars in mm.



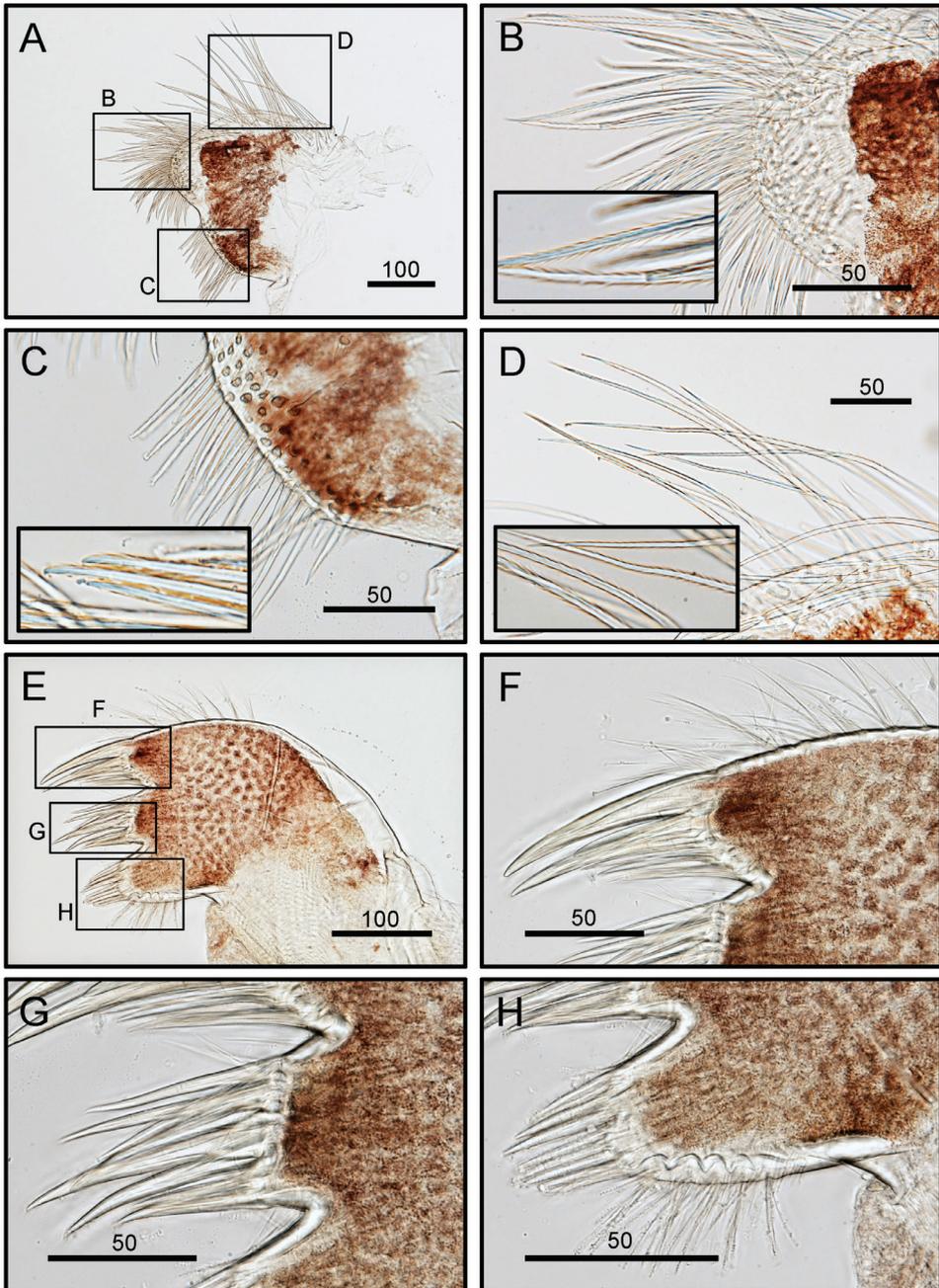
**Figure 5.** *Octomeris brunnea* (CEL-KT-131, Hai Kou, Taiwan). **A** Cirrus I. **B** Posterior ramus of cirrus I. **C** Bidentate serrate setae at tip of segment. **D** Bidentate serrate setae at tip of anterior ramus. **E** Cirrus II. **F** Dorsal side of posterior ramus. **G, H** Bidentate serrate setae at posterior ramus. Scale bars in  $\mu\text{m}$ .



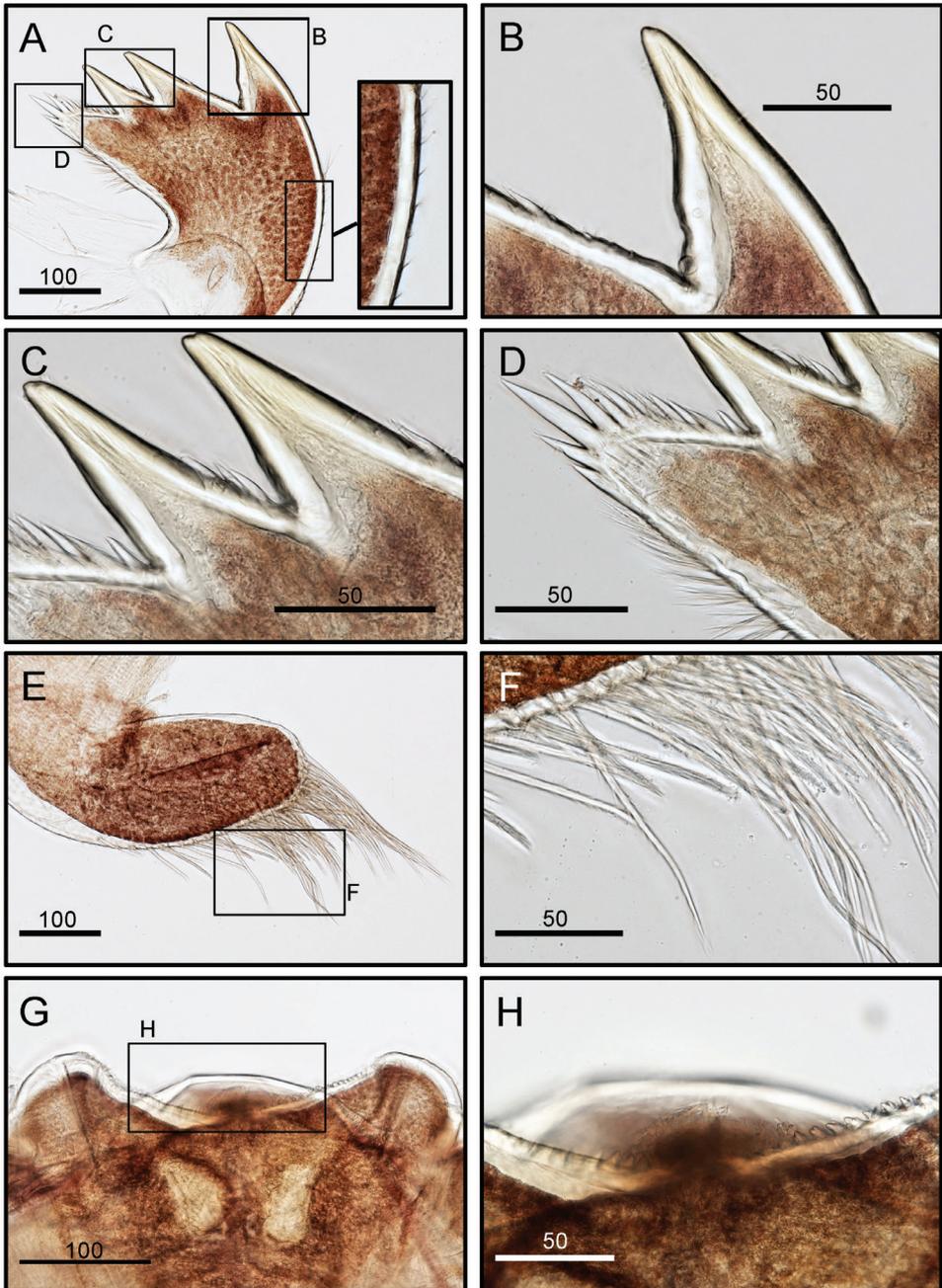
**Figure 6.** *Octomeris brunnea* (CEL-KT-131, Hai Kou, Taiwan). **A** Cirrus III. **B** Intermediate segments of posterior ramus of cirrus III. **C** Distal segments of anterior ramus of cirrus III. **D** Simple type setae on anterior ramus of cirrus III. **E** Cirrus IV. **F** Intermediate segments of cirrus IV. **G** Dorsal surface of proximal segment of posterior ramus of cirrus IV. **H** Distal segments of anterior ramus of cirrus IV. Scale bars in  $\mu\text{m}$ .



**Figure 7.** *Octomeris brunnea* (CEL-KT-131, Hai Kou, Taiwan). **A** Cirrus V. **B** Intermediate segments of posterior ramus of cirrus V. **C** Distal segments of anterior ramus of cirrus V. **D** Cirrus VI. **E** Intermediate segments of posterior ramus of cirrus VI. **F** Dorsal surface of proximal segments of posterior ramus of cirrus VI. **G** Penis. **H** Distal end of penis. Scale bars in  $\mu\text{m}$ .



**Figure 8.** *Octomeris brunnea* (CEL-KT-131, Hai Kou, Taiwan). **A** Maxilla. **B** Magnified view of distal lobe showing serrulate setae. **C** Inner margin of proximal lobe showing serrulate setae. **D** Outer margin of maxilla showing serrulate setae. **E** Maxillule; note the two deep notches on upper and lower 1/3 of the cutting edge. **F** Cutting edge above upper notch. **G** Middle portion of cutting edge. **H** Lower portion of cutting edge below lower notch. Scale bars in  $\mu\text{m}$ .



**Figure 9.** *Octomeris brunnea*. **A** Mandible. **B** First tooth of mandible. **C** Second and third teeth of mandible. **D** Lower margin. **E** Mandibular palp. **F** Serrulate setae at outer margin of mandibular palp. **G** Labrum. **H** Small teeth on middle part of cutting edge of labrum. Scale bars in  $\mu\text{m}$ .

**Distribution.** Southern Japan, Taiwan, Philippines, east coast of Queensland in Australia, Santa Cruz and New Hebrides (based on specimens in Australian Museum stated in Pope, 1965).

***Octomeris intermedia* Nilsson-Cantell, 1921**

Figures 2A–F, 10–14, 15A–C, 16A–C

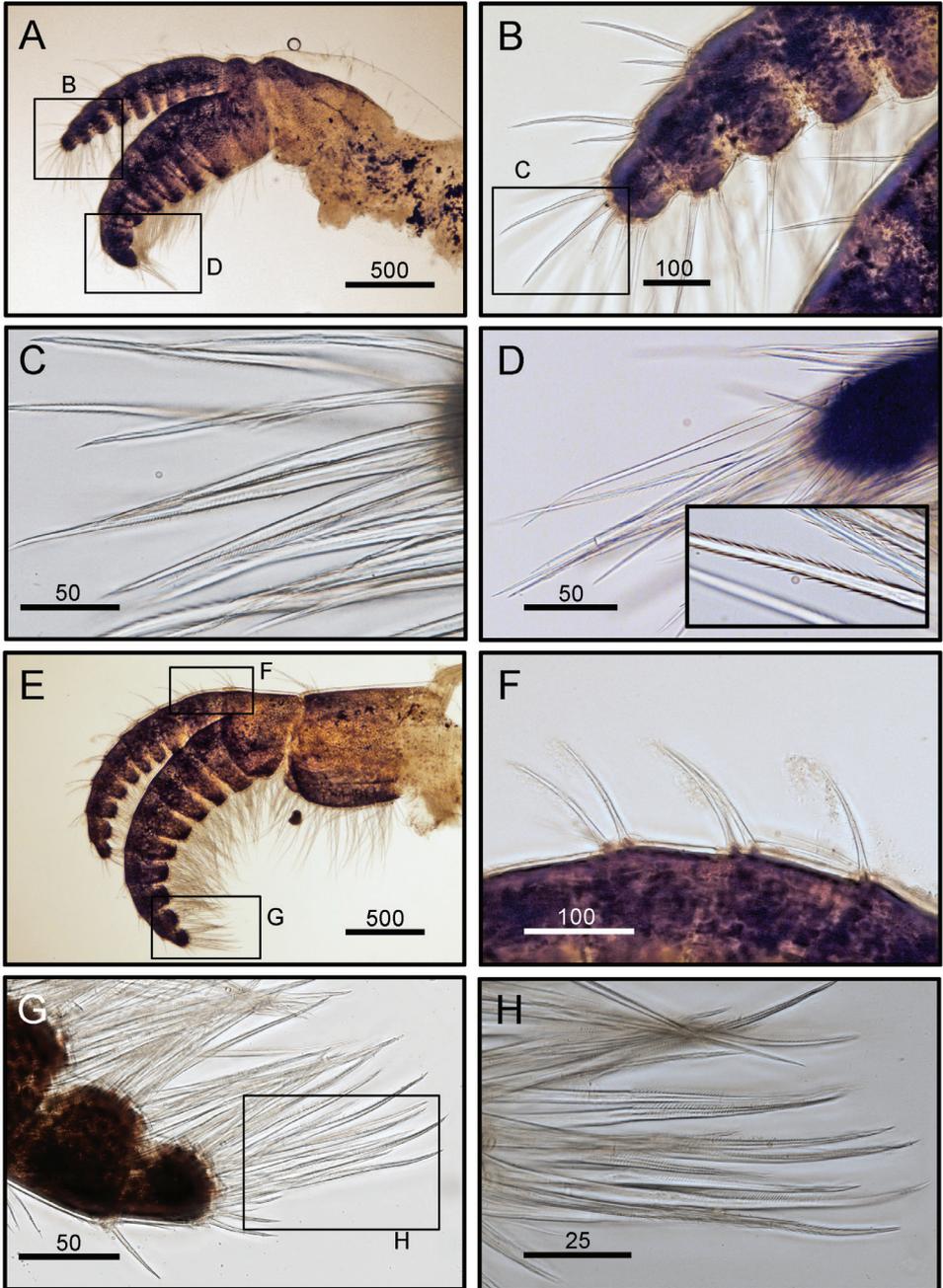
*Octomeris brunnea* Nilsson-Cantell, 1921: 303, figs 60, 61, pl 3, fig. 8.–1925: 1 (erratum for type locality); 1932: 13; 1938: 33, fig. 5; Hiro 1939: 252; Pope 1965: 21; Jones 2012: tabs 1, 2.

**Material examined.** ASIZCR-000431. Intertidal rocks at Ao Nang Beach, Krabi, Thailand (8°02.06'N, 98°48.58'E, 3 July 2019, 1 specimen). CEL-Thai-359. Intertidal rocks at Ao Nang Beach, Krabi, Thailand (8°02.06'N, 98°48.58'E, 3 July 2019, 20 specimens). CEL-Thai-243 Intertidal rocks at Hey Island, Phuket, Thailand (7°44.73'N, 98°22.59'E, 15 May 2019, 103 specimens).

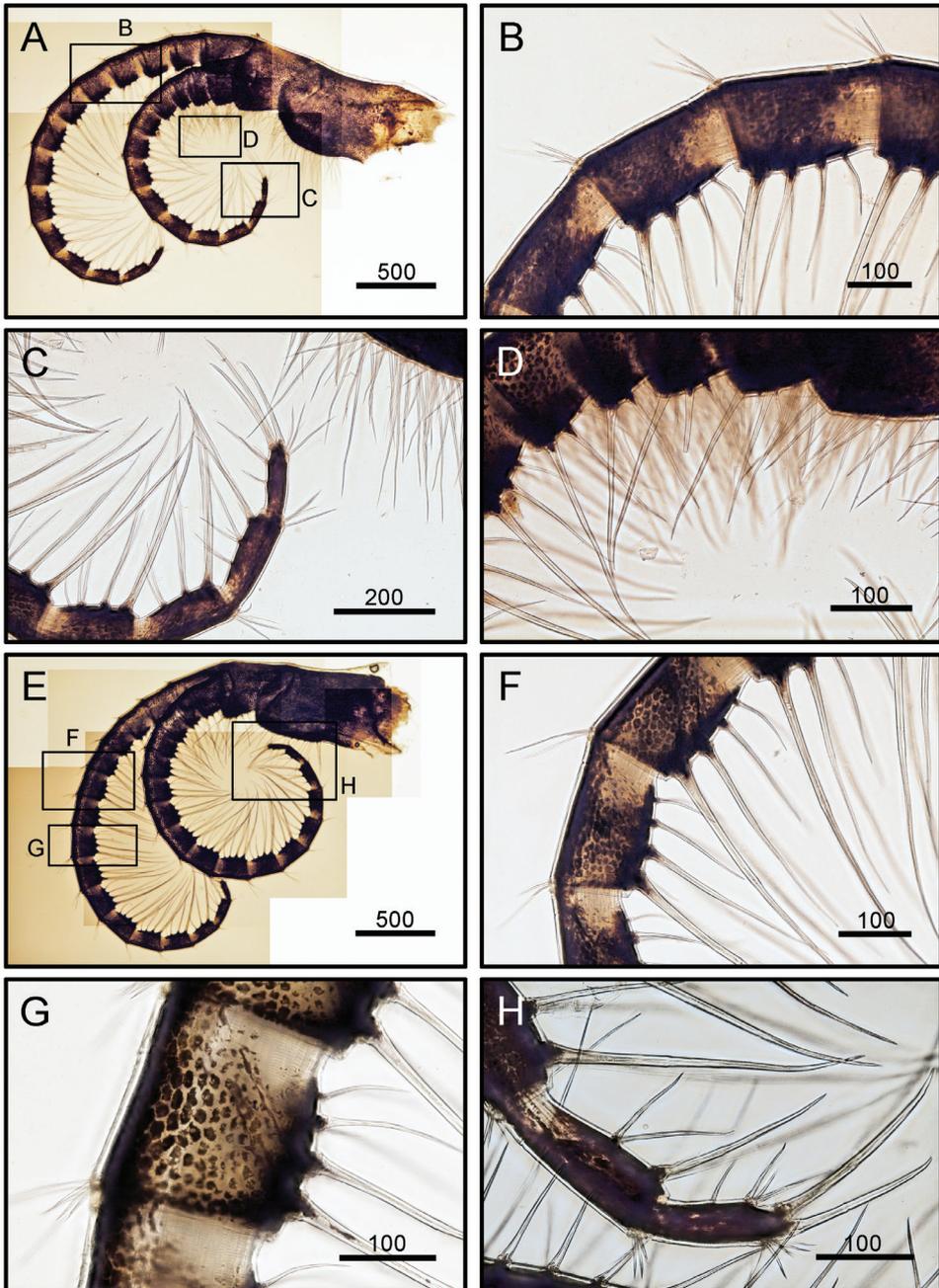
**Diagnosis.** Shell eight plated, very depressed, surface brown with longitudinal furrows on uneroded specimens, tergo-scutal junction sinuous, except for young specimens. Maxillule with very shallow notch at upper 1/3 of cutting edge, lower 1/3 slightly protruded.

**Description.** Shell eight-plated, composed of single rostrum (R) and carina (C), and paired rostro-laterals (RL), carino-laterals (CL) and laterals (L) (Fig. 2D–F). Shell very depressed, brown surface with longitudinal furrows on uneroded specimens (Fig. 2D–F). Tergal-scutal junction sinuous, except for young specimen which has shell length < 10 mm (Fig. 3). Sutures of shell plates serrated (Fig. 4C, D). Scutum triangular, outer surface with horizontal growth lines. Inner surface of scutum brown, occluding margin straight, basal margin slightly convex, tergal margin sinuous with deep articular ridge. Adductor muscle scar shallow (Fig. 4C, D). Basal margin of tergum strongly bended in angle, scutal margin sinuous and with deep articular ridge, crests of depressor muscle crests distinct, muscle crests extended slightly out of the carinal margin of tergum (Fig. 4C, D).

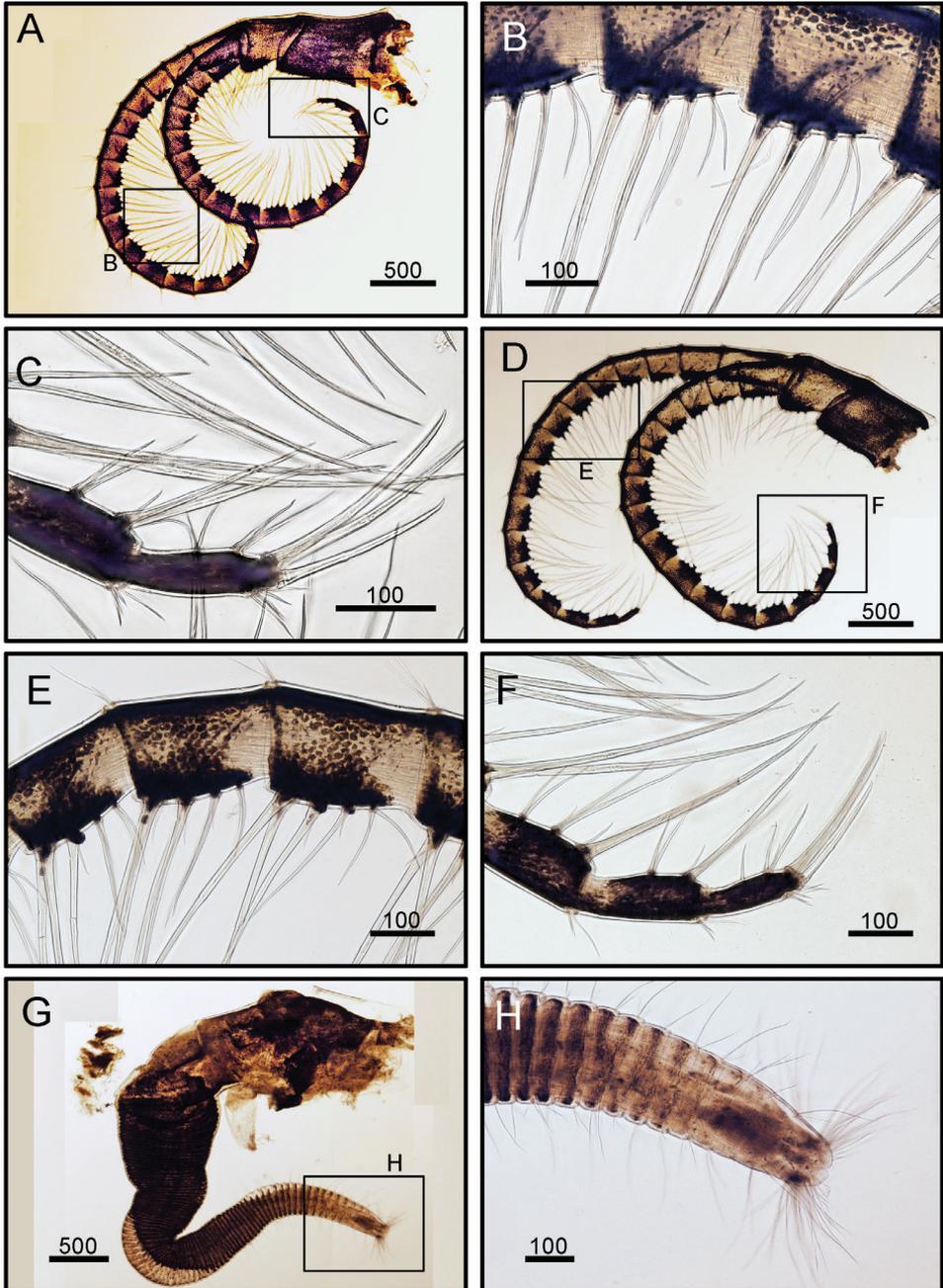
Cirrus I, rami subequal (Fig. 10A–D). Posterior ramus shorter, eight-segmented. Anterior ramus seven-segmented. Segments in both rami with greater height than width. Both rami bear bidentate serrate setae and simple setae (Fig. 10C, D). Bidentate serrate setae present up to seven segments in anterior ramus and present up to first four distal segments in posterior ramus. Cirrus II, posterior ramus nine-segmented, anterior ramus ten-segmented (Fig. 10E, H). Bidentate serrate setae present up to six segments in anterior ramus and nine distal segments in posterior ramus (Fig. 10G, H). Cirri III–VI similar in morphology, being long and slender (Figs 11, 12). Cirrus III, anterior and posterior rami 12-segmented (Fig. 11A–D). Cirrus IV, anterior and posterior rami 16-segmented (Fig. 11E–H). Cirrus V, anterior and posterior rami 17 segmented (Fig. 12A–C). Cirrus VI, anterior ramus 17 segmented and posterior ramus



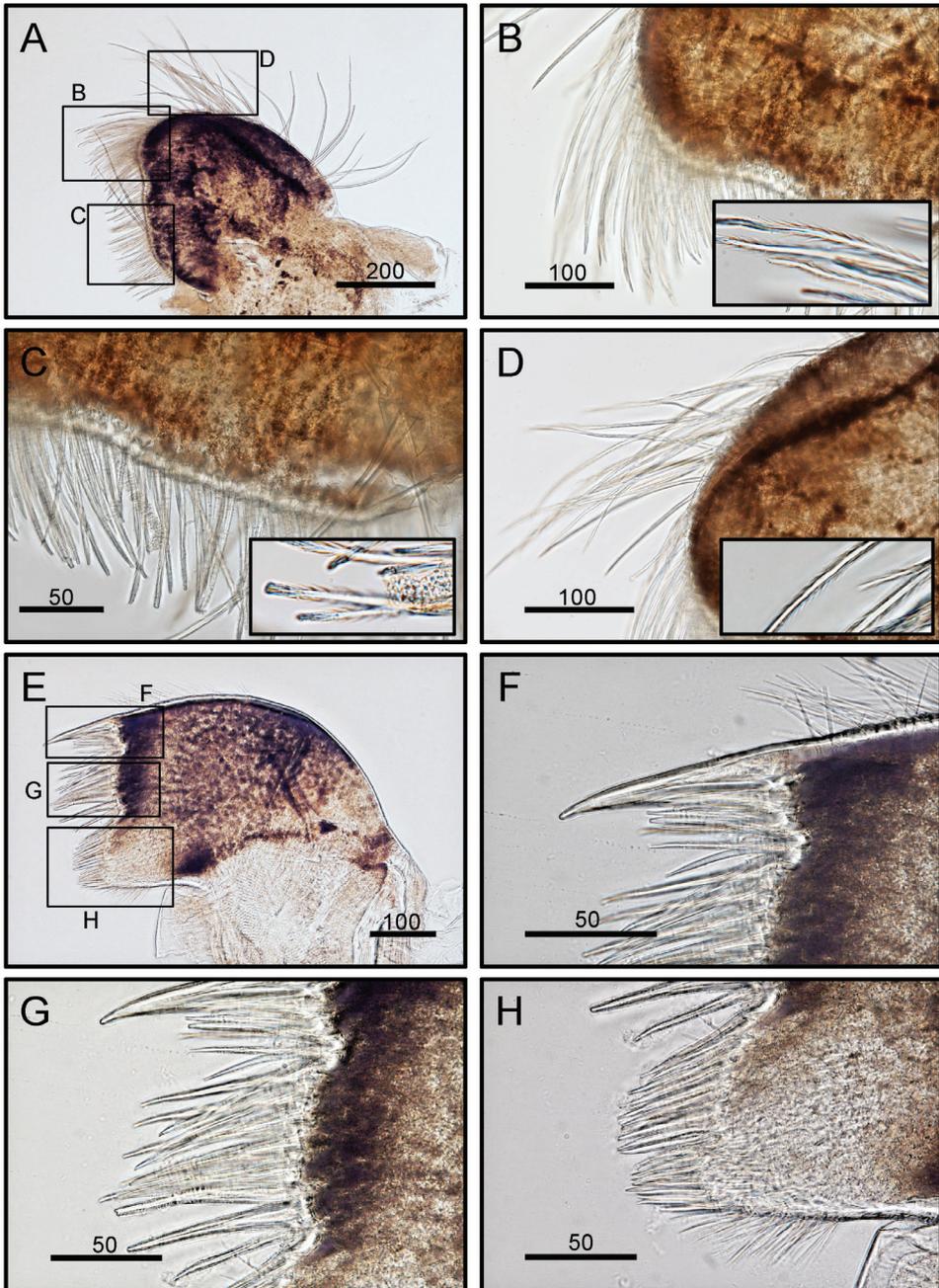
**Figure 10.** *Octomeris intermedia* (CEL-Thai-359, Krabi, Thailand). **A** Cirrus I. **B** Posterior ramus of cirrus I. **C** Bidentate serrate setae at tip of distal segment of posterior ramus. **D** Bidentate serrate setae at tip of anterior ramus. **E** Cirrus II. **F** Dorsal side of posterior ramus. **G, H** Bidentate serrate setae at posterior ramus. Scale bars in  $\mu\text{m}$ .



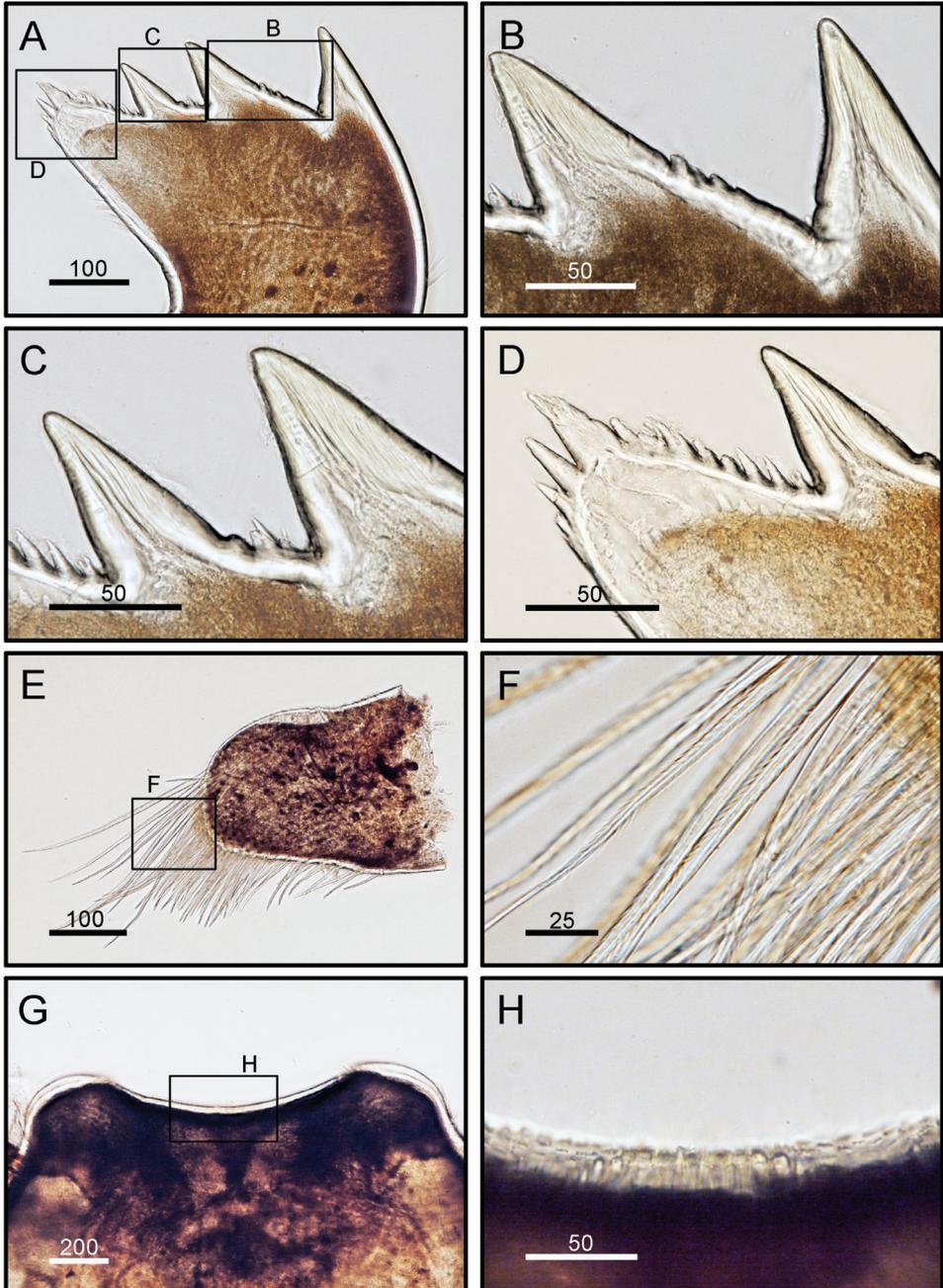
**Figure 11.** *Octomeris intermedia* (CEL-Thai-359, Krabi, Thailand). **A** Cirrus III. **B** Intermediate segments of posterior ramus of cirrus III. **C** Distal segment of anterior ramus of cirrus III. **D** Simple setae at intermediate segments of anterior ramus of cirrus III. **E** Cirrus IV. **F** Intermediate segments of posterior ramus cirrus IV. **G** Intermediate segment of posterior ramus of cirrus IV. **H** distal segment of anterior ramus of cirrus IV. Scale bars in  $\mu\text{m}$ .



**Figure 12.** *Octomeris intermedia* (CEL-Thai-359, Krabi, Thailand). **A** Cirrus V. **B** Intermediate segments of posterior ramus of cirrus V. **C** Distal segments of anterior ramus of cirrus V. **D** Cirrus VI. **E** Intermediate segments of posterior ramus of cirrus VI. **F** Distal segment of anterior ramus of cirrus VI. **G** Penis. **H** Distal end of penis. Scale bars in µm.



**Figure 13.** *Octomeris intermedia* (CEL-Thai-359, Krabi, Thailand). **A** Maxilla. **B** Magnified view of distal lobe showing serrulate setae. **C** Inner margin of maxilla showing serrulate setae. **D** Outer margin of maxilla showing serrulate setae. **E** Maxillule; note the two shallow notches on upper and lower 1/3 of the cutting edge. **F** Cutting edge above upper notch. **G** Middle portion of cutting edge. **H** Lower portion of cutting edge. Scale bars in  $\mu\text{m}$ .



**Figure 14.** *Octomeris intermedia* (CEL-Thai-359, Krabi, Thailand). **A** Mandible, whole view **B** First and second teeth of mandible **C** Second and third teeth of mandible of another specimen. **D** Lower margin of mandible. **E** Distal part of mandibular palp. **F** Serrulate setae at distal margin of mandibular palp. **G** Labrum. **H** Cutting edge of labrum, middle part. Scale bars in  $\mu\text{m}$ .

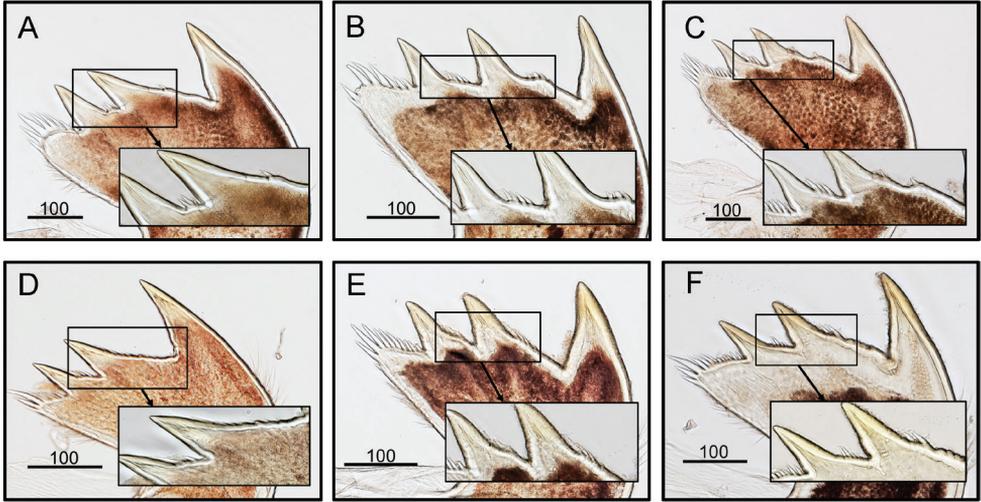
16 segmented (Fig. 12D–F). Intermediate segments of cirri III–VI bear three pairs of long and one pair of short simple setae (Figs 11B, D, F, 12B, E). Distal segments of cirrus III bear two pairs of long and one pair of short setae (Fig. 11C, H; 12C, F). Caudal appendages absent. Penis long, annulated, tip with simple setae (Fig. 12G, H).

Maxilla subtriangular, inner margin with an inconspicuous notch, inner and outer margins with serrulate setae (Fig. 13A–D). Maxillule with two very shallow notches on upper 1/3 and lower 1/3 of cutting edge. Cutting edge more or less straight but the region above notch with one large and a few setae; middle margin has nine setae; cutting edge below upper notch has > 20 short setae (Fig. 13E–H). Mandibles with three teeth, first tooth with smooth edge and second teeth with slightly serrated edge, third tooth with smooth edge but occasionally with some spine on edge region (Figs 14A–D, 15A–C). Mandibular palp elongated, with serrulate setae on outer margin (Fig. 14E, F). Cutting margin of labrum concave, with small fine teeth (Fig. 14G–H).

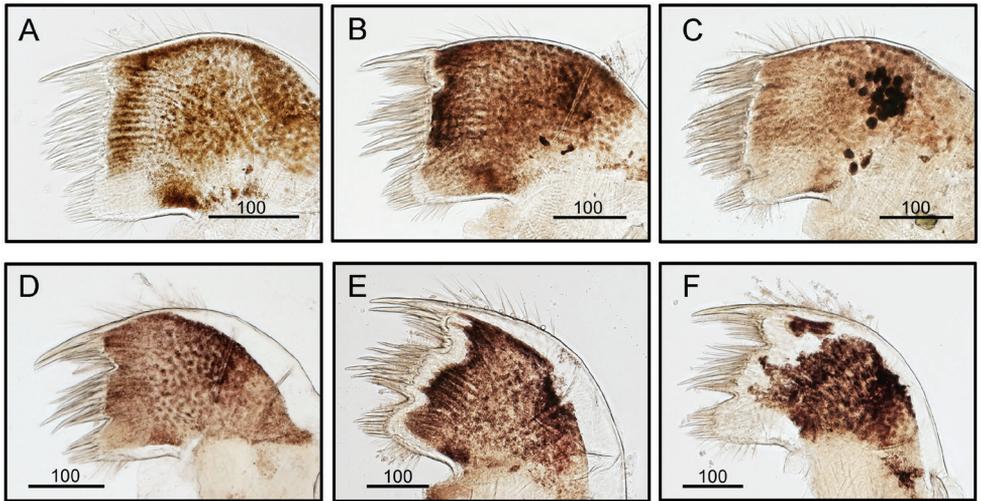
**Distribution.** Java in Indonesia, Mergui Archipelago in Myanmar, Phuket and Krabi in Thailand.

**Remarks.** *O. intermedia* collected in the present study represents the specimens described by Nilsson-Cantell (1921, 1938) who collected *O. intermedia* from Java and the Mergui Archipelago; Phuket and Krabi are approximately 300 km south of the Mergui Archipelago. The morphology of our specimens fits the description in Nilsson-Cantell (1921, 1938): the shell is depressed and has a sinuous junction between the tergum and scutum. The external shell morphology of *O. brunnea* and *O. intermedia* is very similar. Old and eroded specimens of *O. brunnea* have a sinuous junction line between scutum and tergum, which is one of the characteristics of *O. intermedia* described by Nilsson-Cantell (1921). There are, however, several consistent diagnostic features between *O. intermedia* and *O. brunnea*. The shell of *O. intermedia* is much depressed in comparison to that of *O. brunnea*. The junction of the tergum and scutum in *O. intermedia* is sinuous, even at the young stage (except for very small individuals, RC-diameter < 10 mm; Fig. 3). In contrast, young individuals of *O. brunnea* have a straight junction between the scutum and tergum, and this junction line becomes sinuous when the barnacles get older (Fig. 3). There are some variations in the number of spines (–2–4) on the region between the second and third teeth of mandibles in *O. brunnea* and *O. intermedia* (Fig. 15). But both species have similar range of variations and there are no diagnostic differences between the two species (Fig. 15). The maxillule of *O. brunnea* has very deep notches on the upper and lower 1/3 portions of the cutting edge dividing it into three distinct portions. While the maxillule of *O. intermedia* has shallow notches on the upper and lower 1/3 portions of the cutting margin looking more or less straight without being dividing into three distinct regions as in *O. brunnea*. Such differences are considered consistent based on observations of the additional three specimens from both species (Fig. 16).

Nilsson-Cantell (1921) has not state any deposition nor specimen number of type or paratype specimens of *O. intermedia*. The foreword section of Nilsson-Cantell (1921) stated majority of specimens in Nilsson-Cantell (1921) were obtained from collections in Swedish Imperial Museum in Stockholm, Sweden and Zoological Museum in Uppsala, Sweden. The specimens of *O. intermedia* are possibly housed in either one



**Figure 15.** Variation in the occurrence of small spines along the cutting edge of second and third teeth of mandibles in *O. intermedia* (A-C) (CEL-Thai-359, Krabi, Thailand) and *O. brunnea* (D-F) (CEL-KT-131, Hai Kou, Taiwan). Scale bars in  $\mu\text{m}$ .



**Figure 16.** Consistent differences in the presences of shallow and deep notches on the cutting edge of *O. intermedia* (A-C) CEL-Thai-359, Krabi, Thailand) and *O. brunnea* (D-F) (CEL-KT-131, Hai Kou, Taiwan), respectively. The notches in *O. brunnea* are much deeper, and three distinct regions can be seen along the cutting edge. Scale bars in  $\mu\text{m}$ .

of the two museums above. Before checking the presences or absences of *O. intermedia* in museum collections in Sweden, the present study did not attempt to establish any neotypes of *O. intermedia* to avoid taxonomic confusion. The information of the COI gene in GenBank for *O. intermedia* is currently adequate for future studies to confirm identification of specimens collected.

## Molecular analysis

All the phylogenetic results suggested that both *Octomeris brunnea* and *O. intermedia* were clustered their own clades with high bootstrap values and posterior probabilities. The sequences from Fisher et al. (2004) and Pérez-Losada et al. (2012), which were designated as *O. brunnea*, were clustered with *O. intermedia* collected from Malaysia and Thailand by the NJ method (Fig. 17A, B). The phylogeny reconstructed by ML and BI suggested that *O. brunnea* and *O. intermedia* were sister groups (Fig. 18).

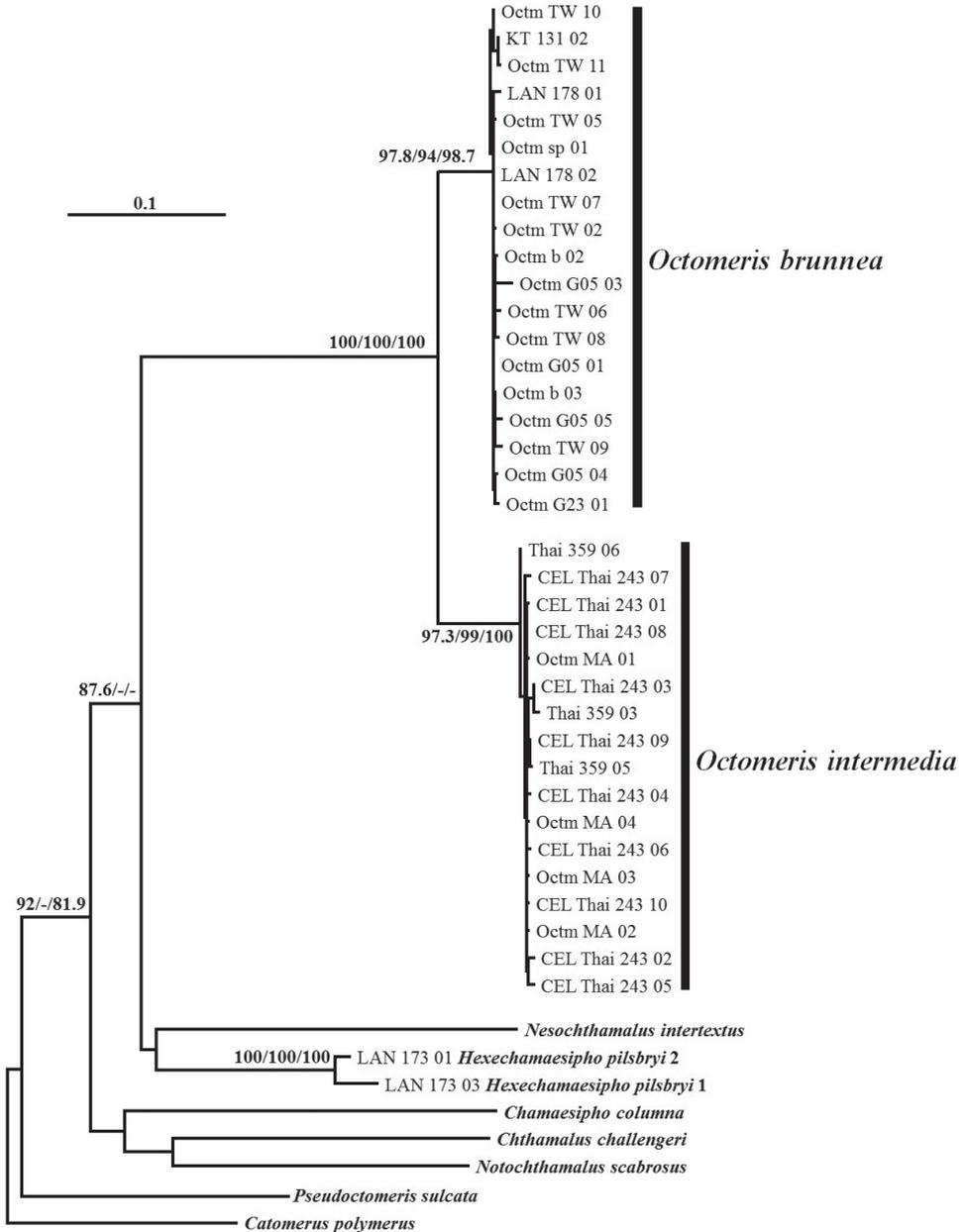
The K2P distances within *O. brunnea* and *O. intermedia* were  $0.007\pm 0.001$  and  $0.005\pm 0.001$  for the COI sequences, and  $0.004\pm 0.001$  and  $0.004\pm 0.001$  for the 12S rRNA sequences, respectively. The K2P distances between *O. brunnea* and *O. intermedia* were  $0.098\pm 0.013$  and  $0.043\pm 0.001$  for the COI and 12S rRNA sequences, respectively. The K2P distances between these two species and other species ranged from 0.207 to 0.251 for the COI sequences and 0.167 to 0.303 for the 12S rRNA sequences (Table 2).

## Discussion

In the present study, we conclude that *Octomeris intermedia* is a valid species using integrative taxonomy. There are consistent morphological differences in the shell and maxillule of *O. intermedia* and *O. brunnea*, suggesting they are two distinct species. *Octomeris intermedia* is common in the west coast, on the Indian Ocean side of the Malay Peninsula. *Octomeris brunnea* is common in the Pacific Ocean and the South China Sea. Molecular analysis suggests that *O. brunnea* and *O. intermedia* are sister clades. However, *O. angulosa* collected by Pérez-Losada et al. (2012) is located outside the clades containing *O. brunnea* and *O. intermedia*. The close relationship between *O. intermedia* and *O. brunnea* in the phylogenetic analysis suggests that these two species may have formed when the Sunda Shelf was exposed during the Pleistocene glaciations, separating the Indian and Pacific Oceans (Voris 2000). Many sister taxa or distinct population genetic divergences in other marine species, including *Tetraclita* and *Chthamalus* barnacles and coral reef fishes, also formed when the Indian and Pacific Oceans separated during the last glacial maxima (Bowen et al. 2001; Tsang et al. 2011, 2012).

Fisher et al. (2004) and Pérez-Losada et al. (2012) included *Octomeris brunnea* in their phylogenetic studies. These *O. brunnea* were collected in Phuket, Thailand. In the phylogenetic analysis in the present study, the sequences of *O. brunnea* of Fisher et al. (2004) and Pérez-Losada et al. (2012) were clustered in the same clade as the *O. intermedia* collected from Phuket and Krabi in the present study, suggesting that these specimens of *O. brunnea* in Fisher et al. (2004) and Pérez-Losada et al. (2012) are *O. intermedia*. *Octomeris angulosa* is recorded from South African waters, and there are no other records outside this region. Fisher et al. (2004) included *Octomeris angulosa* from South Africa (region around the type locality) in their phylogenetic analysis, and the COI gene of this *O. angulosa* is a sister molecular clade with *O. brunnea* and *O. inter-*





**Figure 18.** Maximum likelihood (ML) phylogenetic tree based on the COI and 12S rRNA sequences. The SH-aLRT support, ultrafast bootstrap support, and posterior probability (%) above 80 are represented at the nodes.

**Table 2.** Kimura 2-parameter (K2P) distances of COI and 12S rRNA sequences between species by MEGA X. The lower left of the matrix are the mean distances, and the upper right of the matrix are the SD.

(A) COI												
	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>Octomeris brunnea</i>		0.013	0.021	0.020	0.021	0.021	0.020	0.021	0.021	0.019	0.023	0.022
2. <i>O. intermedia</i>	0.098		0.020	0.020	0.020	0.019	0.022	0.021	0.020	0.020	0.021	0.023
3. <i>O. angulosa</i>	0.209	0.210		0.022	0.021	0.023	0.023	0.023	0.020	0.021	0.021	0.020
4. <i>Chamaesipho tasmanica</i>	0.208	0.215	0.243		0.019	0.019	0.022	0.021	0.020	0.018	0.020	0.022
5. <i>Pseudoctomeris sulcata</i>	0.226	0.231	0.212	0.193		0.019	0.020	0.022	0.020	0.018	0.020	0.020
6. <i>Chamaesipho colamna</i>	0.232	0.218	0.247	0.204	0.207		0.019	0.020	0.020	0.018	0.020	0.021
7. <i>Neochthamalus intertextus</i>	0.211	0.247	0.251	0.219	0.222	0.205		0.022	0.021	0.018	0.019	0.022
8. <i>Microveneraphia rhizophorae</i>	0.246	0.241	0.247	0.221	0.243	0.216	0.224		0.019	0.021	0.023	0.022
9. <i>Chthamalus challengeri</i>	0.237	0.220	0.199	0.196	0.202	0.200	0.220	0.186		0.019	0.019	0.021
10. <i>Hexechamaesipho pilobryi</i>	0.201	0.207	0.199	0.164	0.175	0.179	0.167	0.217	0.195		0.019	0.019
11. <i>Notochthamalus scabrosus</i>	0.241	0.223	0.215	0.212	0.208	0.217	0.196	0.250	0.200	0.189		0.021
12. <i>Catomeris polymerus</i>	0.229	0.251	0.197	0.240	0.195	0.212	0.236	0.238	0.226	0.193	0.226	
(B) 12S rRNA												
	1	2	3	4	5	6	7	8	9	10	11	12.000
1. <i>Octomeris brunnea</i>		0.011	0.030	0.033	0.029	0.031	0.029	0.029	0.029	0.024	0.029	0.028
2. <i>O. intermedia</i>	0.043		0.028	0.036	0.030	0.032	0.030	0.028	0.029	0.024	0.028	0.029
3. <i>O. angulosa</i>	0.239	0.224		0.031	0.027	0.031	0.031	0.025	0.025	0.027	0.026	0.028
4. <i>Chamaesipho tasmanica</i>	0.279	0.303	0.260		0.034	0.018	0.036	0.033	0.036	0.031	0.033	0.032
5. <i>Pseudoctomeris sulcata</i>	0.239	0.248	0.213	0.285		0.030	0.029	0.025	0.031	0.024	0.026	0.027
6. <i>Chamaesipho colamna</i>	0.263	0.275	0.258	0.115	0.246		0.034	0.030	0.033	0.029	0.031	0.029
7. <i>Neochthamalus intertextus</i>	0.238	0.252	0.244	0.299	0.224	0.279		0.030	0.031	0.028	0.029	0.031
8. <i>Microveneraphia rhizophorae</i>	0.239	0.238	0.194	0.276	0.183	0.238	0.230		0.026	0.023	0.025	0.025
9. <i>Chthamalus challengeri</i>	0.223	0.231	0.182	0.301	0.264	0.283	0.245	0.193		0.028	0.028	0.027
10. <i>Hexechamaesipho pilobryi</i>	0.167	0.170	0.207	0.252	0.179	0.239	0.220	0.159	0.210		0.027	0.021
11. <i>Notochthamalus scabrosus</i>	0.220	0.215	0.190	0.281	0.200	0.248	0.228	0.177	0.213	0.204		0.026
12. <i>Catomeris polymerus</i>	0.211	0.219	0.225	0.273	0.206	0.243	0.265	0.200	0.219	0.145	0.200	

*media* in the present study (there are no 12S genes of *O. angulosa* in Fisher et al. 2004). Pérez-Losada et al. (2012) included *Octomeris angulosa* collected in Sydney, Australia in their phylogenetic analysis. According to Pope (1965) and Jones (2012), only *Octomeris brunnea* has been recorded in Australian waters. Only the 12S rRNA sequences of this *O. angulosa* from Pérez-Losada et al. (2004) was available for our analysis. The 12S rRNA sequence of *O. angulosa* from Pérez-Losada et al. (2012) located the taxon outside the clade containing both *O. intermedia* and *O. brunnea* in the present study. Future studies should focus on the diversity and taxonomy of *Octomeris* in Australia.

Intertidal barnacle diversity in Thailand received very little attention until the recent studies of Pochai et al. (2017) and Sukparangsi et al. (2019), who conducted detailed surveys of Thai intertidal barnacles and recorded a total of eleven species from the Thai coastline. The distribution of intertidal barnacles is different between the coastline in the Gulf of Thailand and the Andaman Sea (Pochai et al. 2017): the Andaman side has a higher species diversity (nine total species in Andaman side and six in the Gulf of Thailand). *Octomeris* was not reported by Pochai et al. (2017) or Sukparangsi et al. (2019). The record of *O. intermedia* in the present study brings the number of Thai intertidal barnacle species to 12. No *Octomeris* were found during sampling trips by the first author to Si-Chang Island and Chumporn in the Gulf of Thailand. This suggests that the coastlines in Thai waters that *O. intermedia* is located is probably the Andaman Sea. Therefore, there are ten species of intertidal barnacles on the Andaman side, and six in the Gulf of Thailand.

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# Redescription of *Pristidia cervicornuta* (Araneae, Clubionidae), with a first description of the female

Jianshuang Zhang<sup>1</sup>, Hao Yu<sup>2,4</sup>, Yang Zhong<sup>3,4</sup>

**1** School of Life Sciences, Guizhou Normal University, Guiyang, Guizhou, China **2** School of Biological Sciences, Integrated Mountain Research Institute, Guizhou Education University, Guiyang, Guizhou, China **3** School of Nuclear Technology and Chemistry & Biology, Hubei University of Science and Technology, Xianning, Hubei, China **4** Hubei Collaborative Innovation Center for Green Transformation of Bio-Resources, Centre for Behavioral Ecology and Evolution, College of Life Sciences, Hubei University, Wuhan, Hubei, China

Corresponding author: Hao Yu ([insect1986@126.com](mailto:insect1986@126.com)), Yang Zhong ([hubeispider@aliyun.com](mailto:hubeispider@aliyun.com))

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## Abstract

*Pristidia cervicornuta* Yu, Zhang & Chen, 2017 is redescribed based on new material from the type locality, Diaoluo Mountains of Hainan Island, China. The female is described and illustrated for the first time. In addition, this paper further illustrates the male, and provides a supplementary description.

## Keywords

Diaoluo Mountains, DNA barcoding, morphology, sac spiders, taxonomy

## Introduction

*Pristidia* Deeleman-Reinhold, 2001 is a relatively small genus, distributed exclusively in South East Asia, with only six species described so far, two of which are known from China (Yu et al. 2017; World Spider Catalog 2019). All *Pristidia* species were known from both sexes, except for *P. cervicornuta* Yu, Zhang & Chen, 2017.

*Pristidia cervicornuta* was first described based on two male specimens from Mt. Diaoluo of Hainan Island, China (Yu et al. 2017). Recently new material has been collected from the type locality containing both sexes. The males were identified as *P. cervicornuta* based on comparison with the type specimens. On the basis of the morphological characters (Fig. 1) and DNA barcoding (Table 1), we credibly matched the females and males together as *P. cervicornuta*. Additionally, we found some characters overlooked in the original description of the male. The aim of the current paper is to redescribe the male and report the female for the first time, providing detailed morphological descriptions and illustrations.

## Materials and methods

Specimens were examined with an Olympus SZX7 stereomicroscope; details were studied with an Olympus BX41 compound microscope. Female epigynes and male palps were examined and illustrated after being dissected. Epigynes were removed and cleared in warm lactic acid before illustration. Vulva was also imaged after being embedded in Arabic gum. Photos were made with a Cannon EOS70D digital camera mounted on an Olympus CX41 compound microscope. The digital images were taken and assembled using Helicon Focus 6.80 software package.

All measurements were obtained using an Olympus SZX7 stereomicroscope and given in millimetres. Eye diameters are taken at widest point. The total body length does not include chelicerae or spinnerets length. Leg lengths are given as total length (femur, patella, tibia, metatarsus, tarsus). The terminology used in text and figure legends follows Yu et al. (2017) and Yu et al. (2012).

A DNA barcode was also obtained for matching. A partial fragment of the mitochondrial cytochrome oxidase subunit I (CO1) gene was amplified and sequenced for three specimens, using the primers LCO1490 (5'-GGTCAACAAATCATCATAAA-GATATTGG-3') and C1-N-2776 (5'-GGATAATCA-GAATANCGNCGAGG-3'). For additional information on extraction, amplification and sequencing procedures, see Malumbres-Olarte and Vink (2012). All sequences were analysed using BLAST and are deposited in GenBank. The accession numbers are provided in Table 1.

All specimens (including molecular vouchers) are deposited in the Museum of Guizhou Education University, Guiyang, Guizhou, China (MGEU, curator Hao Yu).

**Table 1.** Voucher specimen information.

Voucher code	Sex	GenBank accession number	Sequence length
MGEU-PRI-18-031 (YHCLU0006)	♂	MN897086	650bp
MGEU-PRI-18-032 (YHCLU0007)	♂	MN897087	650bp
MGEU-PRI-18-017 (YHCLU0008)	♀	MN897088	650bp

## Taxonomy

### Family Clubionidae Wagner, 1887

### Genus *Pristidia* Deeleman-Reinhold, 2001

**Type species.** *Pristidia prima* Deeleman-Reinhold, 2001

**Diagnosis.** For details see Deeleman-Reinhold (2001) and Yu et al. (2017).

**Composition and distribution.** *Pristidia longistila* Deeleman-Reinhold, 2001 from Borneo, *P. prima* Deeleman-Reinhold, 2001 from Thailand, Malaysia and Indonesia (Sumatra, Java), *P. secunda* Deeleman-Reinhold, 2001 endemic to Sumatra, *P. viridissima* Deeleman-Reinhold, 2001 widespread from Thailand to Borneo, *P. ramosa* Yu, Sun & Zhang, 2012 and *P. cervicornuta* from China.

### *Pristidia cervicornuta* Yu, Zhang & Chen, 2017

Figs 1–5

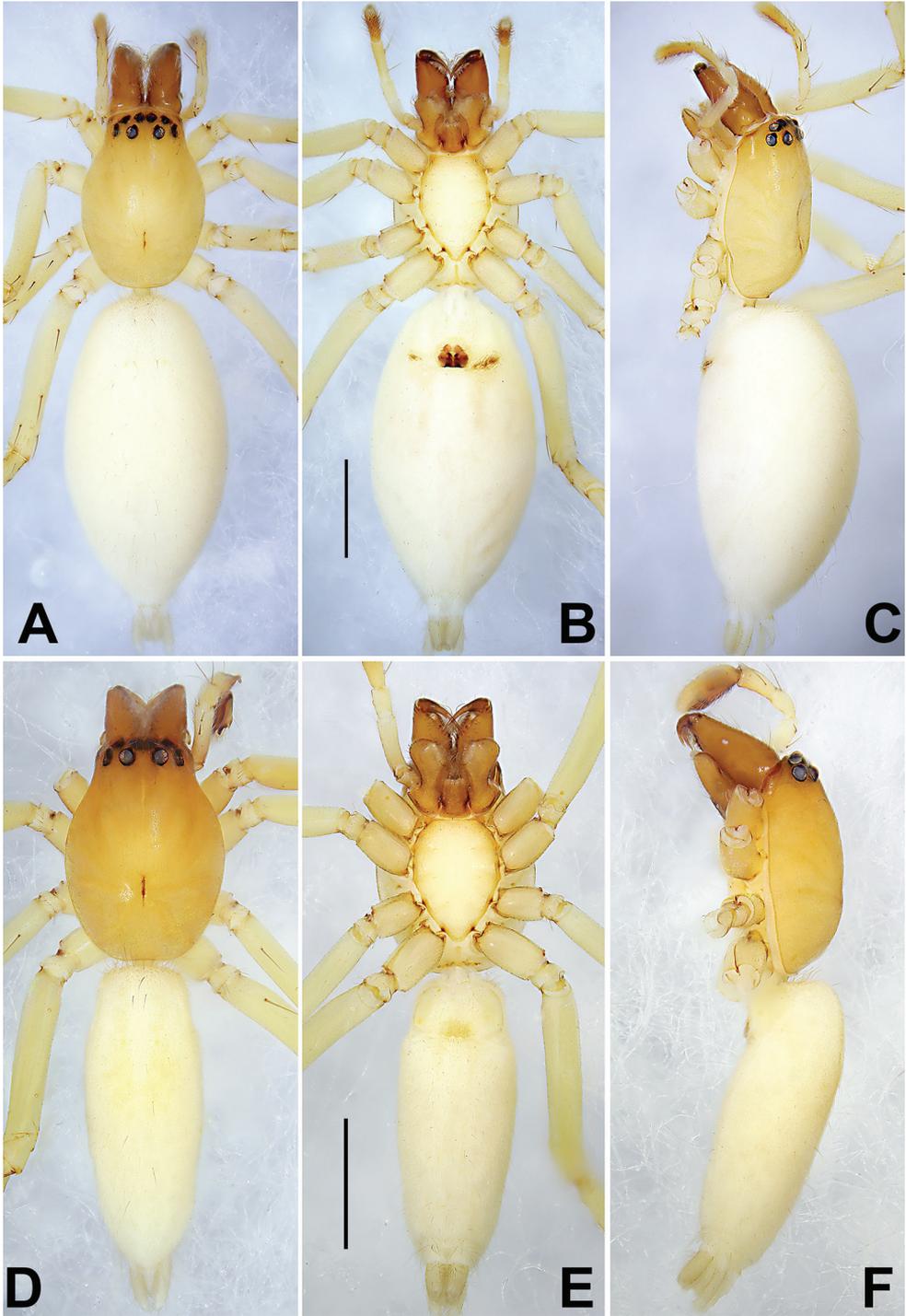
*Pristidia cervicornuta* Yu, Zhang & Chen, 2017: 413, f. 1–8 (♂).

**Material examined.** CHINA • 17♀ (MGEU-PRI-18-001–017) and 15♂ (MGEU-PRI-18-018–032) Hainan Province, Diaoluo Mountains Nature Reserve, Taiping farm; 18°48'15.22"N, 109°52'8.94"E; 380 m; beating of bush, 15 Apr. 2018, Qian Yu leg.; • 2♂ (**Types**); same locality; 18°48'12.16"N, 109°52'5.42"E; 6 Oct. 2009, Hao Yu and Zhenyu Jin leg; beating of shrubs.

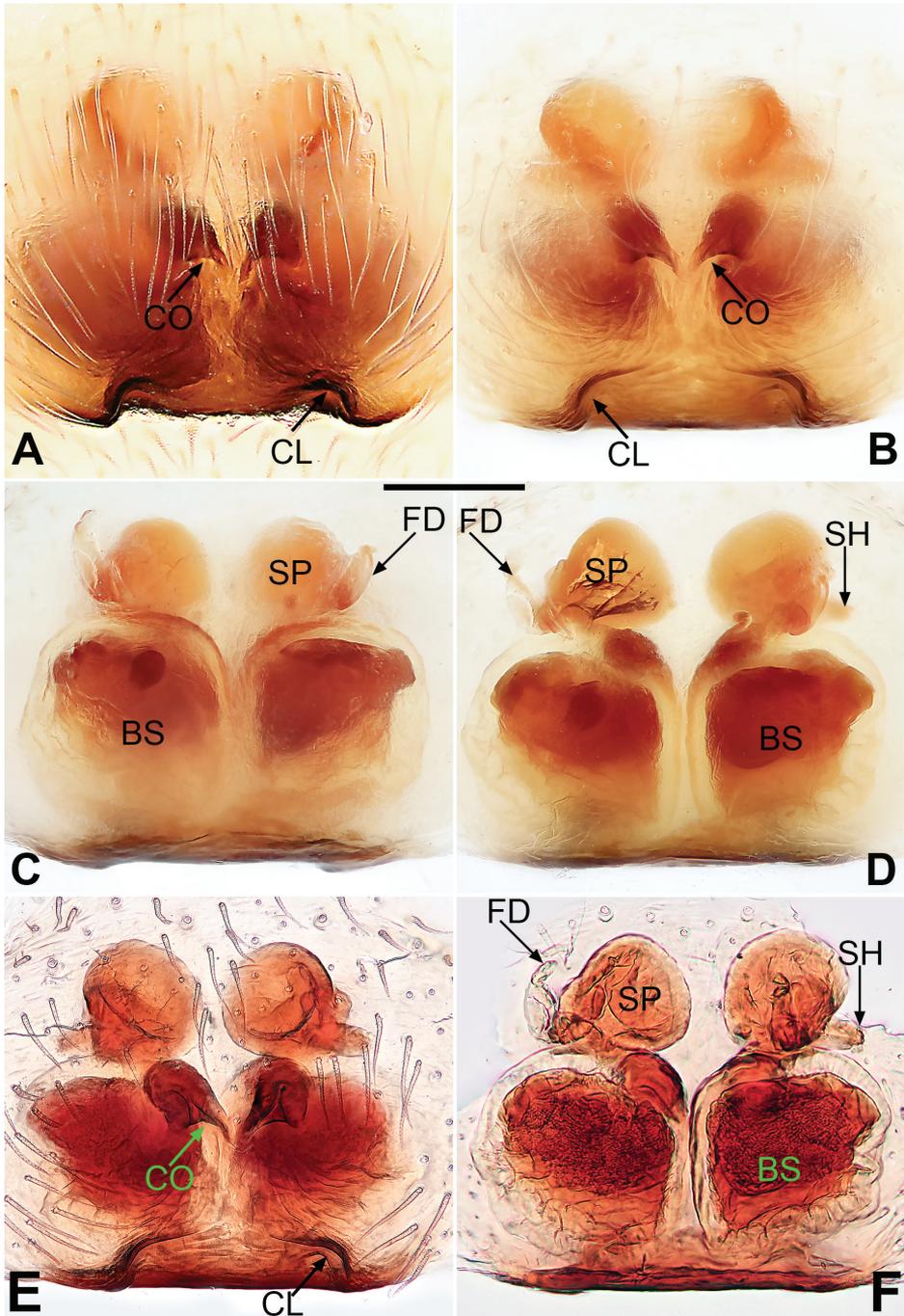
**Diagnosis.** Females of *P. cervicornuta* are similar to those of *P. ramosa* (the other only *Pristidia* species in China: Yu et al. 2012: 45, figs 9–11, 15–16) by the epigynal plate having 2 clefts situated at the posterior margin; they also resemble those of *P. secunda* (Deeleman-Reinhold 2001: 186, figs 191, 192) in the general shape of the vulva, but can be differentiated from *P. ramosa* by lacking atrium (Fig. 2A, B, E) (vs. atrium present in *P. ramosa*), and can be easily distinguished by the indistinct insemination ducts (Fig. 2C, D, F) (vs. long insemination ducts in *P. ramosa* and *P. secunda*), and by the copulatory openings located in the middle of the epigynal plate (Fig. 2A, B, E) (located on posterior margin in *P. ramosa* and *P. secunda*). Males of *P. cervicornuta* can be easily recognized by the distally forked, antler-shaped retrolateral tibial apophysis and by the thick, semitransparent, thumb-shaped tegular apophysis.

**Description. Female** (MGEU-PRI-18-001) (Fig. 1A–C). Total length 5.25; carapace 1.90 long, 1.29 wide; abdomen 3.21 long, 1.65 wide.

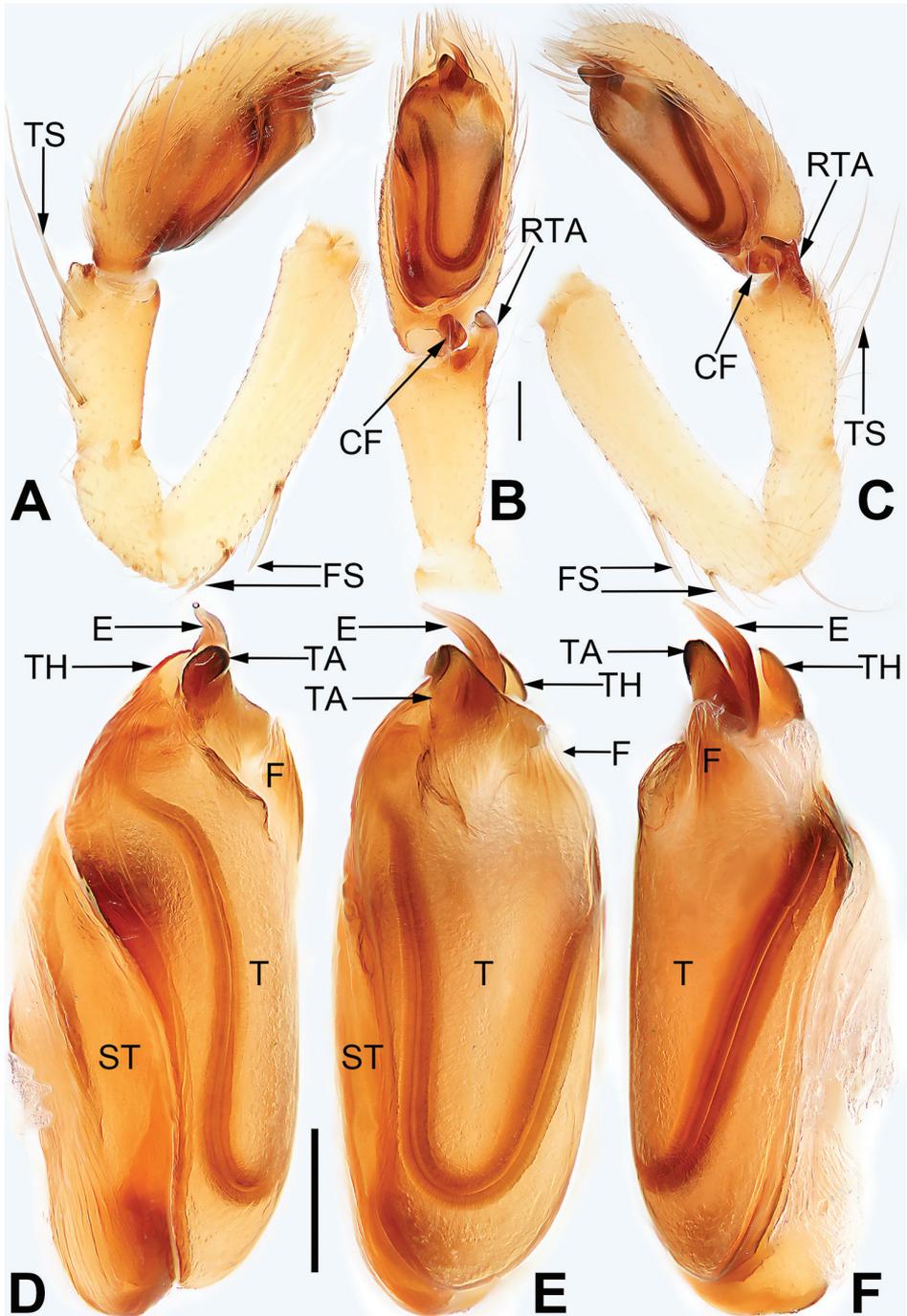
*Carapace* yellow, without distinct pattern. Fovea red. In dorsal view, anterior eye row (AER) slightly recurved, posterior eye row (PER) almost straight, PER wider than AER. Eye sizes and interdistances: anterior median eyes (AME) 0.07, anterior lateral eyes (ALE) 0.05, posterior median eyes (PME) 0.10, posterior lateral eyes (PLE) 0.06;



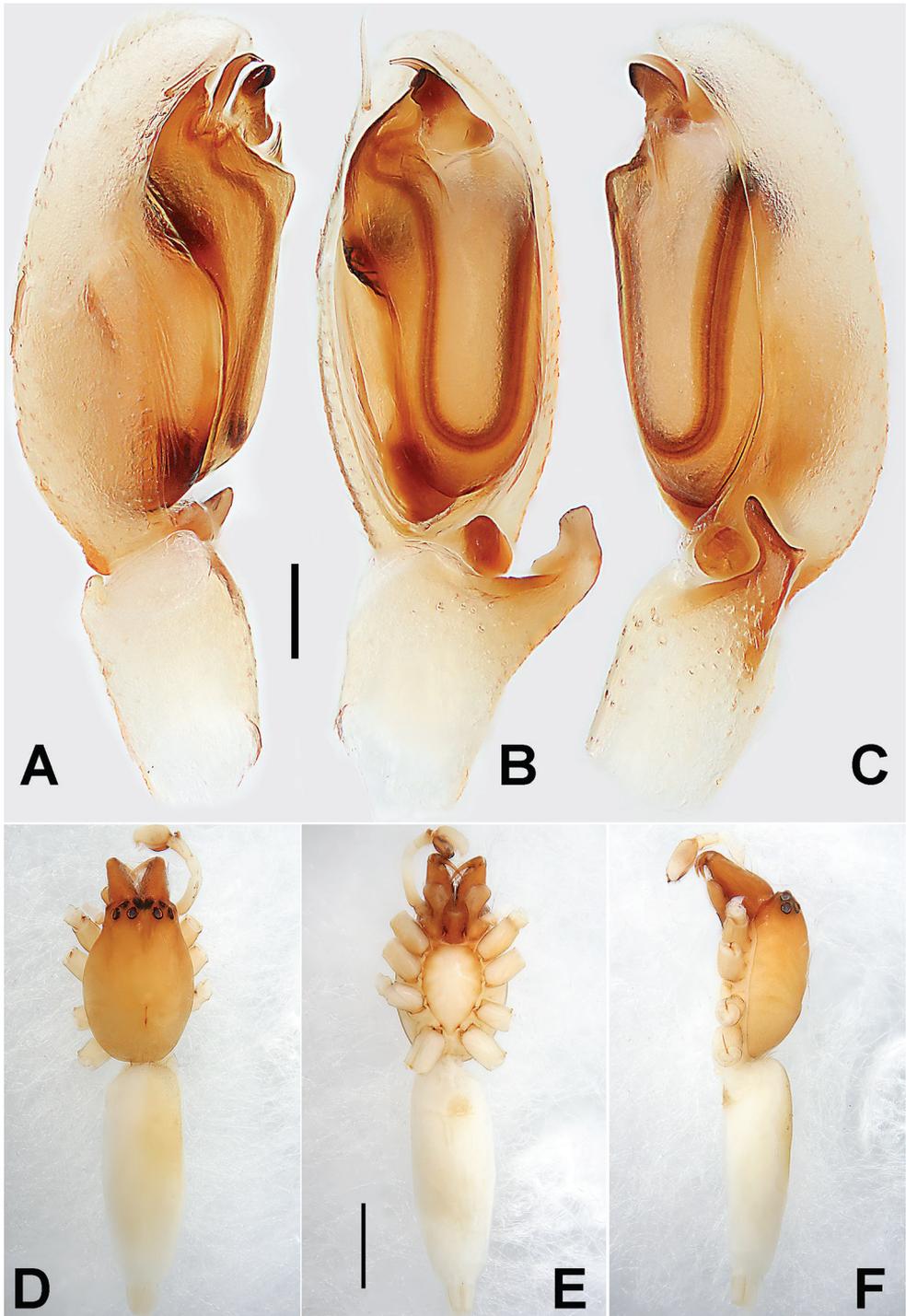
**Figure 1.** Habitus of *Pristidia cervicornuta* female (MGEU-PRI-18-001, **A–C**) and male (MGEU-PRI-18-018, **D–F**). **A, D** Habitus, dorsal view **B, E** ventral view **C, F** Lateral view; Scale bars: 1 mm (equal for **A–C**, equal for **D–F**).



**Figure 2.** *Pristidia cervicornuta*, female (MGEU-PRI-18-002, **A**) and female (MGEU-PRI-18-001, **B-F**). **A** Epigyne, intact, ventral view **B** Epigyne, cleared, ventral view **C** Vulva, cleared, dorsal view **D** Vulva, cleared, dorsal view **E** Epigyne, cleared, ventral view **F** Vulva, cleared, dorsal view. Scale bars: 0.1 mm. Abbreviations: CL, cleft; CO, copulatory opening; FD, fertilization duct; SH, spermathecal head; SP, spermatheca; BS, bursa.



**Figure 3.** Male left palp of *Pristidia cervicornuta* (MGEU-PRI-18-018). **A** Prolateral view **B** Ventral view **C** Retrolateral view **D** Bulb, prolateral view **E** Same, ventral view **F** Same, retrolateral view. Scale bars: 0.1 mm (equal for **A–C**, equal for **D–F**). Abbreviations: CF, cymbial flange; E, embolus; F, flakelet; FS, femoral spines; RTA, retrolateral tibial apophysis; ST, subtegulum; T, tegulum; TA, regular apophysis; TH, regular hump; TS, tibial spines.



**Figure 4.** Holotype male of *Pristidia cervicornuta*. **A** Left palp, prolateral view **B** Same, ventral view **C** Same, retrolateral view **D** Male habitus, dorsal view **E** Same, ventral view **F** Same, lateral view. Scale bars: 0.1 mm (equal for **A-C**); 1 mm (equal for **D-F**).

distance between AMEs (AME–AME) 0.02, distance between AME and ALE (AME–ALE) 0.04, distance between PMEs (PME–PME) 0.13, distance between PME and PLE (PME–PLE) 0.04. Length of median ocular quadrangle (MOQ) 0.26, MOQ anterior width 0.20, MOQ posterior width 0.38. *Chelicerae* protruding and robust, with long and red wine-coloured fangs, with 3 teeth on promargin and 2 on retromargin. Labium and endites brown. Sternum 1.06 long, 0.71 wide.

*Abdomen* lanceolate, white, with inconspicuous anterior tufts of sparse hairs, dorsum without pattern; venter white, with several pairs of inconspicuous muscular dots.

*Legs* uniformly light yellow. Leg length: I 5.13 (1.28, 0.71, 1.75, 0.93, 0.46), II 5.11 (1.43, 0.67, 1.69, 0.81, 0.51), III 4.23 (0.97, 0.49, 1.12, 1.21, 0.44), IV 5.85 (1.57, 0.49, 1.51, 1.75, 0.52).

*Epigyne* (Fig. 2B–F). Epigynal plate slightly shorter than wide, margin not rebordered; posterior margin concaved in the middle, forming shallow depression; 2 clefts located at lateral borders of the depression; copulatory openings distinct and heavily sclerotised, located in the middle of the plate. Insemination ducts short and indistinct; spermathecae located anteriorly; spermathecal head small tubercle-like, located on lateral side of spermatheca; bursal surface hyaline, wrinkled and ribbed, inside pigmented and sclerotised; both spermathecae and bursae are subglobular; acicular fertilisation ducts located on the dorso-lateral sides of spermathecae.

**Male** (MGEU-PRI-18-008) (Fig. 1D–F). Total length 4.06; carapace 1.62 long, 1.28 wide; abdomen 2.25 long, 0.94 wide. Eye sizes and interdistances: AME 0.08, ALE 0.08, PME 0.11, PLE 0.09; AME–AME 0.04, AME–ALE 0.03, PME–PME 0.13, PME–PLE 0.05. MOQL 0.24, MOQA 0.18, MOQP 0.36. Sternum 0.91 long, 0.66 wide. Measurements of legs: I 5.38 (1.26, 0.45, 1.92, 1.16, 0.59), II 5.33 (1.56, 0.45, 1.73, 1.12, 0.47), III 4.39 (1.47, 0.45, 0.76, 1.21, 0.51), IV 6.27 (1.72, 0.54, 1.61, 1.87, 0.54). General characters as in female, but slightly smaller in size and darker in colour.

*Palp* (Fig. 3A–F). See Yu et al. (2017).

**Comments.** There is almost no difference between the holotype male (Fig. 4A–F) and the newly collected male specimen in the present study. However, two characters of the bulb were not presented in the original description. Additionally, some spines and hairs are lost in holotype male (Fig. 4A–C). Consequently, a fuller description is provided here: the tegular hump (TH) is represented by an enlarged flange, hidden behind the embolus (E) and tegular apophysis (TA); a translucent flakelet (F) located at distal-retrolateral position of tegulum (T) (approximately 1 o'clock of tegulum), the flake is subtriangular with a membranous and blunt apex; the tibia has two long dorsal spines (TS) originating from trisection; the femur bears two short dorsal spines (FS) originating from its proximal part.

**Natural history.** *Pristidia cervicornuta* inhabits forest located in low elevation areas on Mt. Diaoluo. The male holotype was obtained from shrubs in a rubber-tea artificial community and the new materials were collected by beating twigs and branches of bush in an elm forest.

**Distribution.** Known only from the type locality, Mt. Diaoluo, Hainan, China (Fig. 5).



**Figure 5.** Type locality of *Pristidia cervicornuta* (red circle).

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# *Labiobaetis* Novikova & Kluge in Borneo (Ephemeroptera, Baetidae)

Thomas Kaltenbach<sup>1,2</sup>, Jean-Luc Gattolliat<sup>1,2</sup>

**1** Museum of Zoology, Palais de Rumine, Place Riponne 6, CH-1005 Lausanne, Switzerland **2** University of Lausanne (UNIL), Department of Ecology and Evolution, CH-1015 Lausanne, Switzerland

Corresponding author: Thomas Kaltenbach ([thomas.kaltenbach@bluewin.ch](mailto:thomas.kaltenbach@bluewin.ch))

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## Abstract

Material collected between 2000 and 2014 on the island Borneo, including the Indonesian province of Kalimantan, the Malaysian province of Sabah and Brunei Darussalam, substantially increased our knowledge of *Labiobaetis* on this island. The total number of *Labiobaetis* species in Borneo increased to five, as only one species, *L. borneoensis* (Müller-Liebenau, 1984), was previously reported. Three new species were identified by morphology and partly by using genetic distance (COI, Kimura 2-parameter). They are described and illustrated based on their larvae (*Labiobaetis bakerae* sp. nov., *L. penan* sp. nov. and *L. dayakorum* sp. nov.); in one case, the imago is described as well. New reports of *L. borneoensis* are presented and the imago of this species is described for the first time. *Labiobaetis moriharai* (Müller-Liebenau, 1984), originally described from mainland Malaysia (Province Selangor), is reported from Borneo for the first time. The interspecific K2P distances in Borneo are between 19% and 25%, the intraspecific distances are usually between 0% and 1%. The total number of *Labiobaetis* species worldwide is augmented to 126.

## Keywords

Brunei, COI, imago, Indonesia, Malaysia, new species, Southeast Asia

## Introduction

The family Baetidae has the highest species diversity among mayflies, comprising 1,070 species in 110 genera (Sartori and Brittain 2015, Jacobus et al. 2019), which is approx. one quarter of all mayfly species worldwide (Gattolliat and Nieto 2009, Jacobus et al.

2019). They have a cosmopolitan distribution except Antarctica and New Zealand. Investigations of the molecular phylogeny of the Order Ephemeroptera revealed the relatively primitive status of the family (Ogden and Whiting 2005, Ogden et al. 2009).

The genus *Labiobaetis* Novikova and Kluge (Novikova and Kluge 1987) is one of the richest genera of Baetidae with previously 123 described species (Barber-James et al. 2013, Webb 2013, Shi and Tong 2014, Kubendran et al. 2014, 2015, Gattolliat et al. 2018, Kaltenbach and Gattolliat 2018, 2019). The distribution of *Labiobaetis* is nearly worldwide, with the exception of the Neotropical realm, New Zealand and New Caledonia. The status and validity of the genus has often been a subject of controversy for a long time, but nowadays *Labiobaetis* is widely accepted as a valid genus (Gattolliat 2001, Fujitani et al. 2003, Fujitani 2008, McCafferty et al. 2010, Gattolliat and Staniczek 2011, Kluge and Novikova 2011, 2014, 2016, Kluge 2012, Webb 2013, Kubendran et al. 2014, 2015, Shi and Tong 2014). The history and concept of the genus *Labiobaetis* were recently summarized in detail (Shi and Tong 2014, Kaltenbach and Gattolliat 2018). All Oriental species previously transferred to *Pseudocloeon* (Lugo-Ortiz et al. 1999) were formerly reassigned to *Labiobaetis* by Shi and Tong (2014). Molecular reconstructions indicated that the concept of *Labiobaetis* is probably at least diphyletic (Monaghan et al. 2005, Gattolliat et al. 2008).

Borneo is the third largest island after Greenland and New Guinea. It forms part of the Sundaland Biodiversity Hotspot comprising Borneo, Sumatra, Java, and the Malay Peninsula and lies at the equator, reaching from 7°N to approx. 4°S, directly West of Wallace's Line (Quek 2010). Borneo belongs to three different countries, the largest part by far in the South and West belongs to Indonesia (Province Kalimantan), another substantial part belongs to Malaysia (Provinces Sabah and Sarawak) and a very small part in the North is Brunei Darussalam. Geomorphically, Borneo is characterised by a central mountain massif with its highest peak, Mt. Kinabalu (4,095 m), in the north, and otherwise, more than half of the island lies below 150 m (Quek 2010). Borneo's biota is very rich, influenced by a dynamic and highly complex geophysical history of the Sunda Shelf, including changing climates, fluctuating sea levels, volcanism and orogenic activity with subsequent erosion (Quek 2010). During an 85 km<sup>2</sup> survey of the mayfly fauna of a lowland tropical forest in Borneo more than 40 mayfly genera were collected and at least ten new genera and many new species were discovered (Derleth 2003, Sartori et al. 2003).

So far, the diversity of *Labiobaetis* in Borneo was poorly known, as only one species was reported (*L. borneoensis* by Müller-Liebenau 1984b). Here, we increase the total number of *Labiobaetis* species in Borneo to five, based on material collected between 2000 and 2014 in ca. 20 different localities, which belong to four different areas in Borneo (Fig. 15). We describe three new species of *Labiobaetis*, one at larval and imaginal stage, the other two based on larvae only. Additionally, we have new reports of *L. borneoensis* (Müller-Liebenau) and we describe the imago of this species for the first time. We also report another species for the first time from Borneo (*L. moribarai*), so far known from mainland Malaysia (Prov. Selangor, Müller-Liebenau 1984a) and Vietnam (Soldán 1991).

## Materials and methods

The specimens from Indonesia (Kalimantan) were collected by Pascale Derleth-Sartori and colleagues (Museum of Zoology Lausanne, MZL; Derleth 2003). Further material was collected by Hendrik Freitag and his team (Ateneo de Manila University), and by Kate Baker (University of Exeter, UK) during ecological studies in Brunei Darussalam in collaboration with Universiti Brunei Darussalam (Baker et al. 2016a, b, 2017a, b).

The specimens were preserved in 70%–96% ethanol. The dissection of larvae was done in Cellosolve (2-Ethoxyethanol) with subsequent mounting on slides with Euparal liquid, using an Olympus SZX7 stereomicroscope.

The DNA of part of the specimens was extracted using non-destructive methods allowing subsequent morphological analysis (see Vuataz et al. 2011 for details). We amplified a 658 bp fragment of the mitochondrial gene cytochrome oxidase subunit 1 (COI) using the primers LCO 1490 (GGTCAACAAATCATAAAGATATTTGG) and HCO 2198 (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994). The polymerase chain reaction was conducted with an initial denaturation temperature of 98 °C for 30 sec followed by a total of 37 cycles with denaturation temperature of 98 °C for 10 sec, an annealing temperature of 50 °C for 30 sec and an extension at 72 °C for 30 sec, final extension at 72 °C for 2 min. Sequencing was done with Sanger's method (Sanger et al. 1977). The genetic variability between specimens was estimated using Kimura 2-parameter distances (K2P, Kimura 1980), calculated with the program MEGA 7 (Kumar et al. 2016, <http://www.megasoftware.net>). The GenBank accession numbers are given in Table 1, nomenclature of gene sequences follows Chakrabarty et al. (2013).

Drawings were made using an Olympus BX43 microscope. Photographs of larvae were taken using a Canon EOS 6D camera and the Visionary Digital Passport imaging system (<http://www.duninc.com>) and processed with the programs Adobe Photoshop Lightroom (<http://www.adobe.com>) and Helicon Focus version 5.3 (<http://www.heliconsoft.com>). Photographs were subsequently enhanced with Adobe Photoshop Elements 13.

The distribution maps were generated with the program SimpleMappr (<https://simplemappr.net> Shorthouse 2010), the program GEOLocate (<http://www.museum.tulane.edu/geolocate/web/WebGeoref.aspx>) and Google Earth (<http://www.google.com/earth/download/ge/>) were used to attribute approximate GPS coordinates to sample locations of Müller-Liebenau (1984a, b) and Soldán (1991).

The taxonomic descriptions were generated with a DELTA (Dallwitz 1980, Dallwitz et al. 1999, Coleman et al. 2010) database containing the morphological states of characters of the *Labiobaetis* species of Borneo.

The terminology follows Hubbard (1995), Morihara and McCafferty (1979), and Kluge (2004). The postero-lateral extension of the paraproct is termed cercotractor following Kluge (2004).

**Table 1.** Sequenced specimens.

Species	Locality		Specimens catalog #	GenBank # (COI)	GenSeq Nomenclature
<i>L. bakerae</i> sp. nov.	Brunei	larva	GBIFCH 00592299	MN482248	genseq-2 COI
		larva	GBIFCH 00658084	MN482250	genseq-2 COI
		larva	GBIFCH 00592282	MN482249	genseq-2 COI
<i>L. penan</i> sp. nov.	Malaysia: Sabah	larva	GBIFCH 00654918	MN482251	genseq-2 COI
		larva	GBIFCH 00672299	MN482252	genseq-1 COI
		imago	GBIFCH 00672296	MN482253	genseq-2 COI
<i>L. borneoensis</i> (Müller-Liebenau)	Malaysia: Sabah	larva	GBIFCH 00658081	MN482254	genseq-4 COI
		imago	GBIFCH 00672289	MN482255	genseq-4 COI
<i>L. moribarae</i> (Müller-Liebenau)	Brunei	larva	GBIFCH 00658106	MN482256	genseq-4 COI

## Results

### New species descriptions

Abbreviations:

**MZL** Museum of Zoology Lausanne (Switzerland)

**PNM** Museum of Natural History of the Philippine National Museum, Manila (Philippines)

### *Labiobaetis sumigarensis* group of species (Müller-Liebenau 1982, Müller-Liebenau and Hubbard 1985, Kaltenbach and Gattolliat 2019)

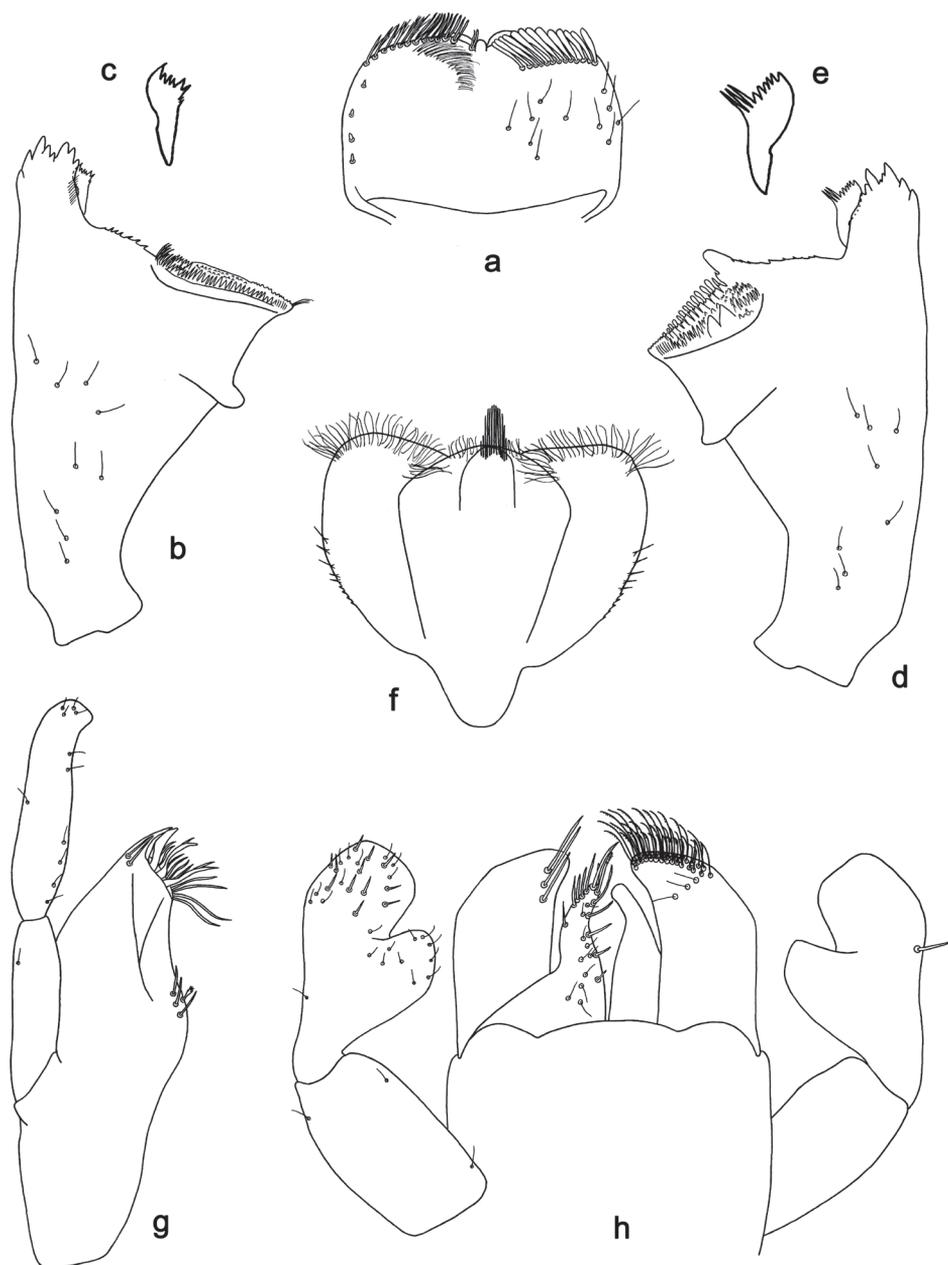
Following combination of characters: A) dorsal surface of labrum with submarginal arc of clavate, apically smooth setae; B) labial palp segment II with large, lobed or thumb-like distomedial protuberance, outer margin of protuberance predominantly concave (*L. sumigarensis* with hook-like modification of the protuberance); C) left mandible without setae at apex of mola, with minute denticles between prostheca and mola; D) six pairs of gills; E) hindwing pads absent; F) distolateral process at scape poorly developed or absent; G) colour of larvae dorsally uniform brown.

### *Labiobaetis bakerae* sp. nov.

<http://zoobank.org/8394FCC0-7343-44F8-B6BF-D06FC34B30C0>

Figures 1, 2, 10a, 14, 15c

**Diagnosis. Larva.** Following combination of characters: A) dorsal surface of labrum with submarginal arc of 13–15 long, clavate setae; B) labial palp segment II with a broad, thumb-like distomedial protuberance, segment III slightly pentagonal; C) left mandible without setae at apex of mola; D) fore femur rather broad, length 3.4× maxi-



**Figure 1.** *Labiobaetis bakerae* sp. nov., larva morphology: **a** Labrum **b** Right mandible **c** Right prostheca **d** Left mandible **e** Left prostheca **f** Hypopharynx **g** Maxilla **h** Labium.

num width, dorsal margin with 8–11 curved, spine-like setae; E) paraproct distally expanded, with 34–39 marginal, stout spines.

**Description. Larva** (Figs 1, 2, 10a). Body length 3.5–4.3 mm; antennae and cerci broken.

*Colouration.* Head, thorax and abdomen dorsally brown; head and thorax with bright median, dorsal suture. Head, thorax and abdomen ventrally light brown; femur ecru, with brown dorsal margin and brown ventrodistomedial spot, tibia and tarsus brown, caudal filaments ecru.

*Antenna* (Fig. 2g) with scape and pedicel subcylindrical, with poorly developed distolateral process at scape.

*Labrum* (Fig. 1a). Rectangular, length  $0.6\times$  maximum width. Distal margin with medial emargination and a small process. Dorsally with medium, fine, simple setae scattered over surface; submarginal arc of setae composed of 13–15 long, clavate setae. Ventrally with marginal row of setae composed of anterolateral long, feathered setae and medial long, bifid setae; ventral surface with four short, spine-like setae near lateral and anterolateral margin.

*Right mandible* (Fig. 1b, c). Incisors fused. Outer and inner sets of denticles with  $4 + 3$  denticles and one minute intermediate denticle. Inner margin of innermost denticle with a row of thin setae. Prosthema robust, apically and distolaterally denticulate. Margin between prosthema and mola straight, with minute denticles. Tuft of setae at apex of mola present.

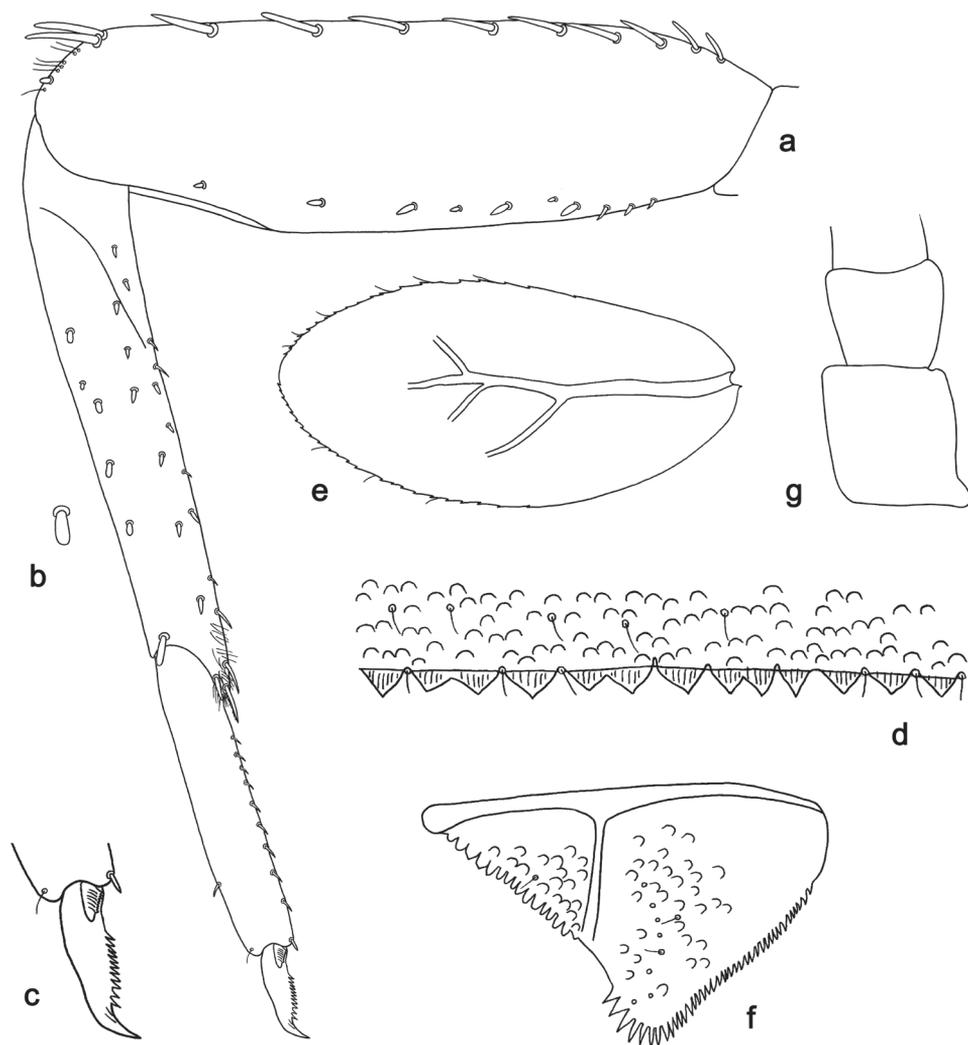
*Left mandible* (Fig. 1d, e). Incisors fused. Outer and inner sets of denticles with  $4 + 3$  denticles and one minute intermediate denticle. Prosthema robust, apically with small denticles and comb-shaped structure. Margin between prosthema and mola straight, with minute denticles towards subtriangular process. Subtriangular process long and slender, above level of area between prosthema and mola. Denticles of mola apically constricted. Tuft of setae at apex of mola absent.

Both mandibles with lateral margins almost straight. Basal half with fine, simple setae scattered over dorsal surface.

*Hypopharynx* (Fig. 1f). Lingua approx. as long as superlingua. Lingua longer than broad; medial tuft of stout setae well developed; distal half laterally expanded. Superlingua straight; lateral margin rounded; fine, long, simple setae along distal margin.

*Maxilla* (Fig. 1g). Galea-lacinia with two simple, robust apical setae under crown. Inner dorsal row of setae with three denti-setae, distal denti-seta tooth-like, middle and proximal denti-setae slender, bifid and pectinate. Medially with one bipectinate, spine-like seta and 3–4 medium, simple setae. Maxillary palp  $1.5\times$  as long as length of galea-lacinia; 2-segmented; palp segment II  $1.5\times$  length of segment I; setae on maxillary palp fine and simple, scattered over surface of segments I and II; apex of last segment rounded, with excavation at inner distolateral margin.

*Labium* (Fig. 1h). Glossa basally broad, narrowing toward apex; shorter than paraglossa; inner margin with five spine-like setae increasing in length distally; apex with two long and one medium, robust, pectinate setae; outer margin with five long, spine-like setae; ventral surface with fine, simple, scattered setae. Paraglossa sub-rectangular, curved inward; apex rounded; with three rows of long, robust, distally pectinate setae in apical area and two or three medium, simple setae in anteromedial area; dorsally with a row of three long, spine-like setae near inner margin. Labial palp with segment I  $0.8\times$  length of segments II and III combined. Segment I with fine, simple setae along



**Figure 2.** *Labiobaetis bakerae* sp. nov., larva morphology: **a** Foreleg **b** Tibia dorsal seta **c** Fore claw **d** Tergum IV **e** Gill IV **f** Paraproct **g** Antennal scape.

margins. Segment II with broad, thumb-like distomedial protuberance; distomedial protuberance  $0.6\times$  width of base of segment III; inner and outer margins with short, fine, simple setae; dorsally with one or two long, spine-like seta near outer margin. Segment III slightly pentagonal; apex rounded; length  $1.1\times$  width; ventrally covered with short, spine-like, simple setae and short, fine, simple setae.

*Hindwing pads* absent.

*Foreleg* (Fig. 2a, b, c). Ratio of foreleg segments  $1.3:1.0:0.6:0.2$ . *Femur*. Length ca.  $3\times$  maximum width. Dorsal margin with a row of 8–11 curved, spine-like setae; length of setae  $0.29\times$  maximum width of femur. Apex rounded; with one pair of curved, spine-like setae, one or a few short stout setae and some fine, simple setae. Stout, lanceolate

setae scattered along the ventral margin; femoral patch absent. *Tibia*. Dorsal margin with a row of stout, apically rounded setae, apically one longer, apically rounded seta. Ventral margin with a row of curved, spine-like setae, on apex a few stout, spine-like, partly bipectinate setae and a tuft of fine, simple setae. Anterior surface scattered with stout, lanceolate setae. Patellotibial suture present on basal 1/3 area. *Tarsus*. Dorsal margin almost bare. Ventral margin with a row of curved, spine-like setae. Tarsal claw with one row of 9–11 denticles; distally pointed; with two stripes; subapical setae absent.

*Terga* (Fig. 2d). Surface with rows of U-shaped scale bases and scattered fine, simple, setae. Posterior margin of tergum IV with triangular spines, wider than long.

*Gills* (Fig. 2e). Present on segments II - VII. Margin with small denticles intercalating fine simple setae. Tracheae partly extending from main trunk towards outer and inner margins. Gill IV as long as length of segments V and 1/3 VI combined. Gill VII as long as length of segments VIII and 1/4 IX combined.

*Paraproct* (Fig. 2f). Distally expanded, with 34–39 stout marginal spines. Surface scattered with U-shaped scale bases, fine simple setae and micropores. Cercotractor with small marginal spines.

**Etymology.** Dedicated to Dr. Kate Baker (University of Exeter, UK), who collected the specimens in Brunei.

**Distribution.** Brunei (Fig. 15c).

**Biological aspects.** The specimens were collected in pools of small lowland forest streams at an altitude of 100 m (Fig. 14).

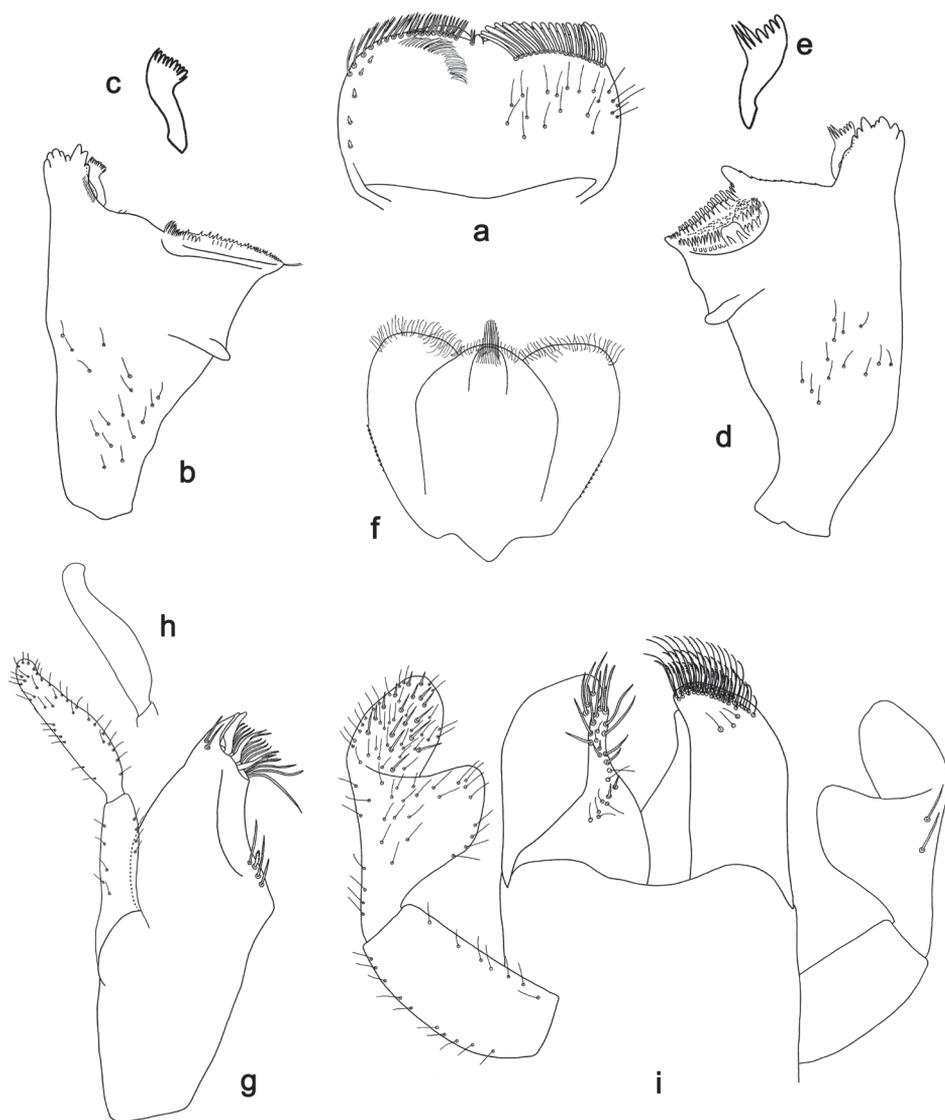
**Type-material. Holotype.** Larva (on slide, GBIFCH 00592236), Brunei, Temburong District, Ulu Temburong National Park, Belalong River (near field station), 04°33.07'N, 115°09.41'E, 100 m, V. 2014, K. Baker leg. Deposited in MZL. **Paratypes.** 2 larva (on slides, GBIFCH 00658097, GBIFCH 00658084), same data as holotype; 5 larvae (on slides, GBIFCH 00592299, GBIFCH 00592296, GBIFCH 00592282, GBIFCH 00284241, GBIFCH 00592298), Brunei, Temburong District, Ulu Temburong National Park, 04°32.77'N, 115°09.52'E, V. 2014, K. Baker leg.; 3 larvae (on slides, GBIFCH 00592297, GBIFCH 00592295, GBIFCH 00592294), Brunei, Temburong District, Ulu Temburong National Park, 04°32.92'N, 115°09.45'E, V. 2014, K. Baker leg. All material deposited in MZL.

***Labiobaetis penan* sp. nov.**

<http://zoobank.org/18DC8E3B-D831-415B-8F97-D3AC264A8931>

Figures 3, 4, 10b, 12a, 13a, c, 15d

**Diagnosis. Larva.** Following combination of characters: A) dorsal surface of labrum with submarginal arc of 18–22 clavate setae; B) labial palp segment II with a broad, thumb-like distomedial protuberance, segment III oblong; C) left mandible without setae at apex of mola; D) fore femur rather broad, length 3.4× maximum width, dorsal margin with a row of 15–19 curved, spine-like setae; E) paraproct distally expanded, with 27–33 marginal, stout spines, some of them with split tips.



**Figure 3.** *Labiobaetis penan* sp. nov., larva morphology: **a** Labrum **b** Right mandible **c** Right prostheca **d** Left mandible **e** Left prostheca **f** Hypopharynx **g** Maxilla **h** Labium.

**Description. Larva** (Figs 3, 4, 10b). Body length 3.8–6 mm. Cerci: approx. as long as body length. Terminal filament: approx. as long as 1/2 length of cerci. Antenna: approximately 3× as long as head length.

**Colouration.** Head, thorax, and abdomen dorsally brown; head and thorax with bright, median, dorsal suture. Head, thorax, and abdomen ventrally light brown, legs light brown, caudal filaments light brown.

**Antenna** (Fig. 4i) with scape and pedicel subcylindrical, without distolateral process at scape.

*Labrum* (Fig. 3a). Rectangular, length 0.6× maximum width. Distal margin with medial emargination and a small process. Dorsally with medium, fine, simple setae scattered over surface; submarginal arc of setae composed of 18–22 long, clavate setae. Ventrally with marginal row of setae composed of lateral and anterolateral long, feathered setae and medial long, bifid setae; ventral surface with five short, spine-like setae near lateral and anterolateral margin.

*Right mandible* (Fig. 3b, c). Incisors fused. Outer and inner sets of denticles with 4 + 3 denticles and one minute intermediate denticle. Inner margin of innermost denticle with a row of thin setae. Prosthema robust, apically denticulate. Margin between prosthema and mola slightly convex, with a few minute setae. Tuft of setae at apex of mola present.

*Left mandible* (Fig. 3d, e). Incisors fused. Outer and inner sets of denticles with 4 + 3 denticles and one minute intermediate denticle. Prosthema robust, apically with small denticles and comb-shaped structure. Margin between prosthema and mola straight, with minute denticles towards subtriangular process. Subtriangular process long and slender, above level of area between prosthema and mola. Denticles of mola apically constricted. Tuft of setae at apex of mola absent.

Both mandibles with lateral margins almost straight. Basal half with fine, simple setae scattered over dorsal surface.

*Hypopharynx* (Fig. 3f). Lingua approx. as long as superlingua. Lingua approx. as broad as long; medial tuft of stout setae well developed; distal half not expanded. Superlingua rounded; lateral margin rounded; fine, long, simple setae along distal margin.

*Maxilla* (Fig. 3g, h). Galea-lacinia with two simple, robust apical setae under crown. Inner dorsal row of setae with three denti-setae, distal denti-seta tooth-like, middle and proximal denti-setae slender, bifid and pectinate. Medially with one bipectinate, spine-like seta and three medium, simple setae. Maxillary palp 1.4× as long as length of galea-lacinia; 2-segmented. Palp segment II 1.4× length of segment I. Setae on maxillary palp fine and simple, scattered over surface of segments I and II. Apex of last segment rounded, with strong excavation at inner distolateral margin.

*Labium* (Fig. 3i). Glossa basally broad, narrowing toward apex; shorter than paraglossa; inner margin with five spine-like setae increasing in length distally; apex with two long and one medium, robust, pectinate setae; outer margin with four long, spine-like setae; ventral surface with short, fine, simple and short, spine-like setae. Paraglossa sub-rectangular, curved inward; apex rounded; with three rows of long, robust, distally pectinate setae in apical area and three medium, simple setae in anteromedial area; dorsally with a row of three long, spine-like setae near inner margin. Labial palp with segment I 0.7× length of segments II and III combined. Segment I ventrally with short, fine, simple setae. Segment II with broad, thumb-like distomedial protuberance; distomedial protuberance 1.0× width of base of segment III; inner and outer margin with short, fine, simple setae; dorsally with two long, spine-like, simple setae near outer margin. Segment III oblong; apex rounded; length 1.4× width; ventrally covered with short to medium, spine-like, simple setae and short, fine, simple setae.

*Hindwing pads* absent.

*Foreleg* (Fig. 4a–d). Ratio of foreleg segments 1.1:1.0:0.4:0.1. *Femur*. Length ca. 3× maximum width. Dorsal margin with a row of 15–19 curved, spine-like, apically rounded setae and many long, fine, simple setae and partly a few stout setae near margin; length of setae 0.28× maximum width of femur. Apex rounded; with one pair of curved, spine-like setae and some short, stout setae. Many stout, lanceolate setae scattered along ventral margin; femoral patch poorly developed. *Tibia*. Dorsal margin with a row of stout, lanceolate, apically rounded setae and fine, simple setae; on apex one larger, lanceolate, apically rounded seta. Ventral margin with a row of curved, spine-like setae, on apex one bipectinate, spine-like seta and a tuft of long, fine, simple setae. Anterior surface scattered with stout, lanceolate setae. Patellotibial suture present on basal 1/3 area. *Tarsus*. Dorsal margin with a row of small, stout setae and fine, simple setae. Ventral margin with a row of curved, spine-like setae. Tarsal claw with one row of 9–11 denticles; distally pointed; with three stripes; subapical setae absent.

*Terga* (Fig. 4e, f). Surface with rows of U-shaped scale bases. Posterior margin of tergum IV with triangular or rounded spines, wider than long.

*Gills* (Fig. 4g). Present on segments II - VII. Margin with small denticles intercalating both short and medium, fine, simple setae. Tracheae extending from main trunk to inner and outer margins. Gill IV as long as length of segments V and 1/3 VI combined. Gill VII as long as length of segment VIII.

*Paraproct* (Fig. 4h). Distally expanded, with 27–33 stout marginal spines, some of them with split tips. Surface scattered with U-shaped scale bases, fine, simple setae and micropores. Cercotractor with small marginal spines.

**Description. Male imago** (Fig. 12a, 13a, c). Body length 3.8 mm, forewing length 4.4 mm.

*Colouration*. Head light beige. Turbinate eyes orange, shaft proximally lighter. Thorax light beige with lateral brown markings (Fig. 13c). Legs light brown. Wings hyaline, venation hyaline. Abdomen dorsally whitish with lateral orange brown markings (Fig. 13c), segment VII dorsally orange brown.

*Forewing* (Fig. 12a). Pterostigma with three cross-veins, distal one bifurcated and reaching subcostal vein, in the middle a short one not reaching subcostal vein and the proximal one reaching subcostal vein; double intercalary veins generally shorter than distance between corresponding main veins at wing margin.

*Hindwing* absent.

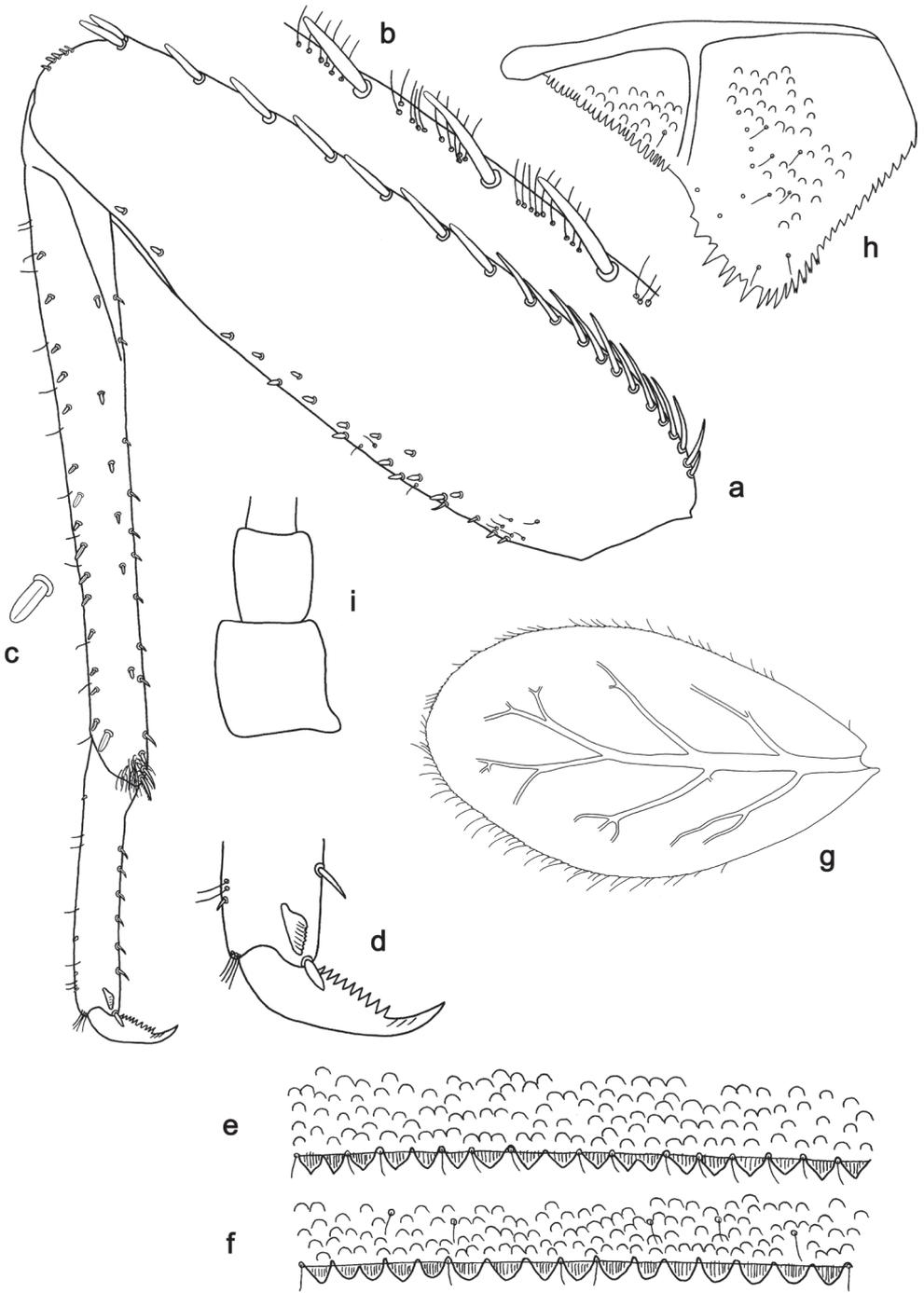
*Genitalia* (Fig. 13a). Basal segment of gonostylus (unistyliger) with inner margin apically only slightly expanded; segments I and II almost completely fused; constriction at base of segment II; segment III ovoid. Styliger plate between unistyligers poorly developed, distal margin straight.

**Etymology**. Dedicated to the indigenous Penan people of Borneo.

**Distribution**. Indonesia: Kalimantan, Brunei, Malaysia: Sabah (Fig. 15d).

**Biological aspects**. The specimens were collected in small, shallow forest streams at altitudes from 100 m to 1,450 m, partly in leaf packs.

**Ontogenetic association**. With genetics, one male imago shares an identical COI sequence with two larvae from the same location (K2P 0%; Table 3).



**Figure 4.** *Labiobaetis penan* sp. nov., larva morphology: **a** Foreleg **b** Femur dorsal setae **c** Tibia dorsal seta **d** Fore claw **e, f** Tergum IV **g** Gill IV **h** Paraproct **i** Antennal scape.

**Type-material. Holotype.** Larva (on slide, GBIFCH 00672299), Malaysia, Sabah, creek near Kundasang, sec. forest, 06°00.40'N, 116°32.80'E, 1450 m, 15.III.2008, Mendoza leg., deposited in PNM. **Paratypes.** 2 larvae (on slides, GBIFCH 00654918, GBIFCH 00592242), same data as holotype; 2 male imagos (1 in alcohol and wing on slide, GBIFCH 00672296, GBIFCH 00606853, 1 in alcohol, GBIFCH 00515330), same data as holotype. All paratypes deposited in MZL. **Other material.** 2 larvae (1 on slide, GBIFCH 00592252, 1 in alcohol, GBIFCH 00658087), Brunei, Temburong District, Ulu Temburong National Park, Belalong River (near field station), 04°32.82'N, 115°09.50'E, 100 m, V. 2014, K. Baker leg.; 1 larva (in alcohol, GBIFCH 00515373), Brunei, Temburong District, Ulu Temburong National Park, Sungai Mata Ikan (tributary to Belalong River, small creek near station), 04°32.83'N, 115°09.38'E, 110 m, V. 2014, K. Baker leg.; 5 larvae (1 on slide, GBIFCH 00592239, 4 in alcohol, GBIFCH 00515327), Brunei, Temburong District, Ulu Temburong National Park, Belalong River tributary, 04°32.63'N, 115°08.85'E, 170 m, V. 2014, K. Baker leg.; 1 larva (on slide, GBIFCH 00592286), Indonesia, East Kalimantan, Bas. Malinau, River Rian, loc. Langap South (1999-block 24), tributary, 03°01.67'N, 116°31.08'E, 11.VII.2000, P. Derleth leg.; 20 larvae (1 on slide, GBIFCH 00592283, 19 in alcohol, GBIFCH 00515396, GBIFCH 515385, GBIFCH 00515386, GBIFCH 00515388, GBIFCH 00515294, GBIFCH 00515314), Indonesia, East Kalimantan, Bas. Malinau, River Rian, loc. Langap South (1997-block 6), trib. Belakau, 03°04.07'N, 116°30.43'E, 05.VII.2000, P. Derleth leg.; 3 larvae (1 on slide, GBIFCH 592284, 2 in alcohol, GBIFCH 00515397), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (2001-block 57), trib. Tamalang, 10.IV.2001, P. Derleth leg.; 18 larvae (1 on slide, GBIFCH 00592251, 17 in alcohol, GBIFCH 00515304, GBIFCH 00515390, GBIFCH 00515302, GBIFCH 00515387), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (2001-block 57), trib. Bengahau, 02°59.37'N, 116°30.77'E, 08.VIII.2000, P. Derleth leg., 1 larva (in alcohol, GBIFCH 00515389), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (1999-block 39-40), trib. Temalat (Sungai Guang), 03°00.17'N, 116°32.40'E, 01.VII.2000, P. Derleth leg.; 9 larvae (in alcohol, GBIFCH 00515305), Indonesia, East Kalimantan, Bas. Malinau, River Rian, Langap South (1995), trib. Ngayo, 03°04.93'N, 116°30.97'E, 13.VII.2000, P. Derleth leg.; 8 larvae (in alcohol, GBIFCH 515301), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (2001-block 57), trib. Tamalang, 19.VII.2000, P. Derleth and F. Béboux leg.; 2 larvae (in alcohol, GBIFCH 00515320), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (2000-block 45), trib. Wok (Sungai Guang), 03°00.15'N, 116°32.42'E, 29.VI.2000, P. Derleth leg.; 7 larvae (in alcohol, GBIFCH 00515293), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (2000-block 44-45), trib. Wok (Sungai Guang), 02°59.20"N 116°33.18'E, 17.VI.2000, P. Derleth and J.-L. Gattolliat leg.; 1 larva (in alcohol, GBIFCH 00515318), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (2000-block 44-45), trib. Wok (Sungai Guang), 02°59.20'N, 116°33.18'E, 16.VI.2000, P. Derleth and J.-L. Gattol-

liat leg.; 13 larvae (in alcohol, GBIFCH 00515300, GBIFCH 00515298, GBIFCH 00515311, GBIFCH 00515295), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (2000-block 43), trib. Temalat (Sungai Guang), 02°59.48'N, 116°33.48'E, 16.VIII.2000, P. Derleth and R. Schlaepfer leg.; 1 larva (in alcohol, GBIFCH 00515384), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (1998-block 28), trib. Kipah, 03°01.80'N, 116°01.80'E, 29.III.2001, P. Derleth leg.; 2 larvae (1 on slide, GBIFCH 00592287, 1 in alcohol, GBIFCH 00515319), Indonesia, East Kalimantan, Bas. Malinau, River Rian, loc. Seturan (1998-block 32–33), tributary, 03°00.95'N, 116°32.27'E, 23.VI.2000, P. Derleth and J.-L. Gattolliat leg.; 4 larvae (in alcohol, GBIFCH 00515303), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (1999-block 27), tributary, 03°00.95'N, 116°30.52'E, 10.VII.2000, P. Derleth leg. All material deposited in MZL.

### ***Labiobaetis operosus* group of species (Kaltenbach and Gattolliat 2019)**

Following combination of characters: A) dorsal surface of labrum with submarginal arc of feathered setae; B) labial palp segment II with thumb-like or lobed distomedial protuberance; C) seven pairs of gills; D) hindwing pads well developed; E) distolateral process at scape well developed.

### ***Labiobaetis dayakorum* sp. nov.**

<http://zoobank.org/A0B3DDF0-8270-4379-9BD8-D0CE90D43EE3>

Figures 5, 6, 11a, 15c

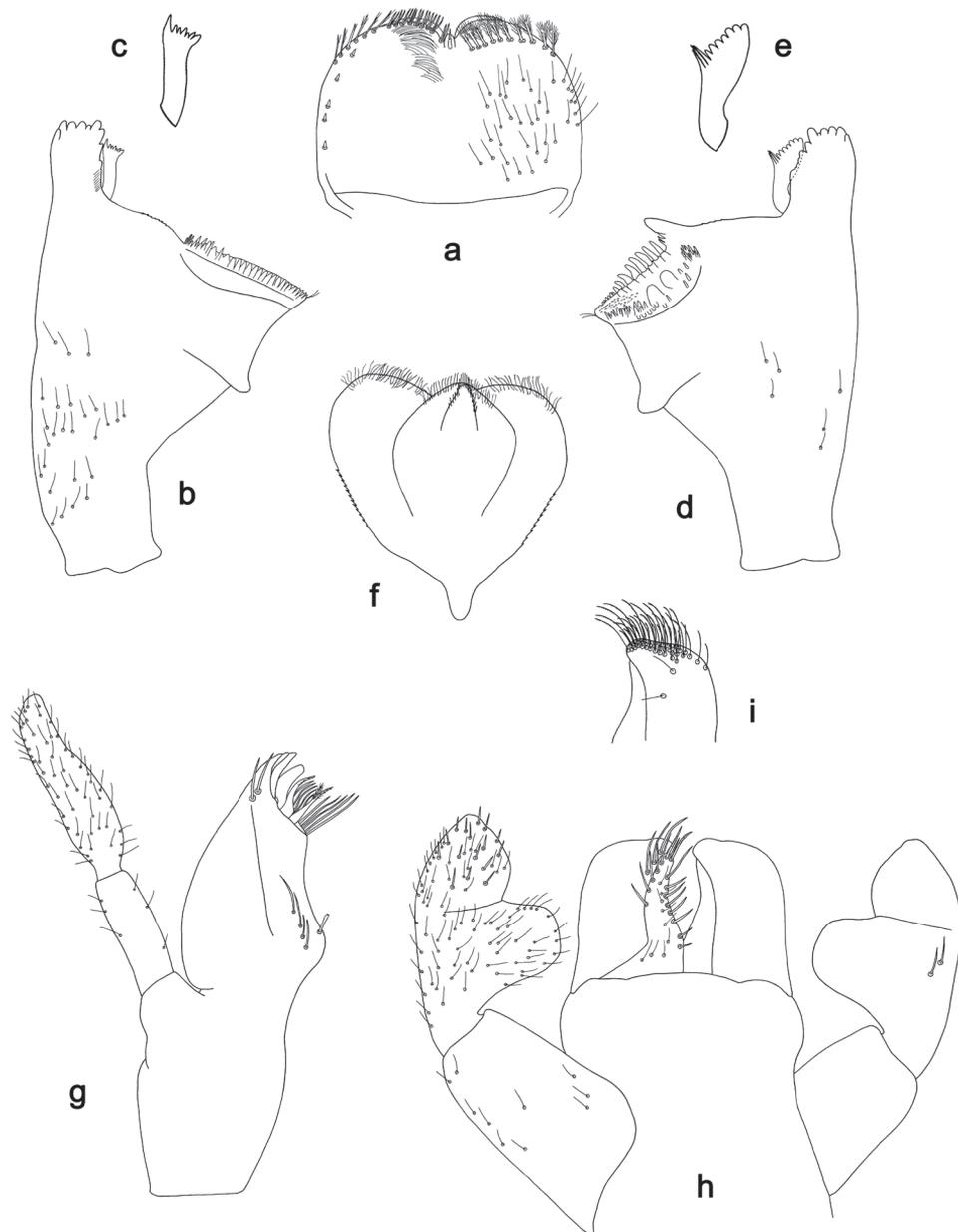
**Diagnosis. Larva.** Following combination of characters: A) dorsal surface of labrum with submarginal arc of 10–12 long, feathered setae; B) labial palp segment II with a large, lobed distomedial protuberance, segment III slightly pentagonal; C) fore femur rather broad, length ca. 4× maximum width, dorsal margin with a row of 12–14 curved, spine-like setae; D) hindwing pads well developed; E) paraproct distally not expanded, with 30–37 marginal, stout spines.

**Description. Larva** (Figs 5, 6, 11a). Body length 5.2 mm; antenna: approximately 2.5× as long as head length; cerci broken.

**Colouration.** Head, thorax and abdomen dorsally brown; head and thorax with bright median, dorsal suture, abdominal segment X light brown. Head, thorax and abdomen ventrally light brown, legs light brown with a brown spot medially and apically on femur, caudal filaments light brown.

**Antenna** (Fig. 6g) with scape and pedicel subcylindrical, with well-developed distolateral process at scape.

**Labrum** (Fig. 5a). Rectangular, length 0.7× maximum width. Distal margin with medial emargination and a small process. Dorsally with medium to long, fine, simple setae scattered over surface; submarginal arc of setae composed of 10–12 long, feath-



**Figure 5.** *Labiobaetis dayakorum* sp. nov., larva morphology: **a** Labrum **b** Right mandible **c** Right prosthema **d** Left mandible **e** Left prosthema **f** Hypopharynx **g** Maxilla **h** Labium **i** Apex of paraglossa.

ered setae. Ventrally with marginal row of setae composed of anterolateral long, feathered setae and medial long, bifid setae; ventral surface with five short, spine-like setae near lateral and anterolateral margin.

*Right mandible* (Fig. 5b, c). Incisors fused. Outer and inner sets of denticles with 4 + 3 denticles and one minute intermediate denticle. Inner margin of innermost denticle

with a row of thin setae. Prosthema robust, apically denticulate. Margin between prosthema and mola slightly convex, with minute denticles. Tuft of setae at apex of mola present.

*Left mandible* (Fig. 5d, e). Incisors fused. Outer and inner sets of denticles with 4 + 3 denticles and one minute intermediate denticle. Prosthema robust, apically with small denticles and comb-shaped structure. Margin between prosthema and mola straight, with minute denticles towards subtriangular process. Subtriangular process long and slender, above level of area between prosthema and mola. Denticles of mola apically constricted. Tuft of setae at apex of mola present.

Both mandibles with lateral margins almost straight. Basal half with fine, simple setae scattered over dorsal surface.

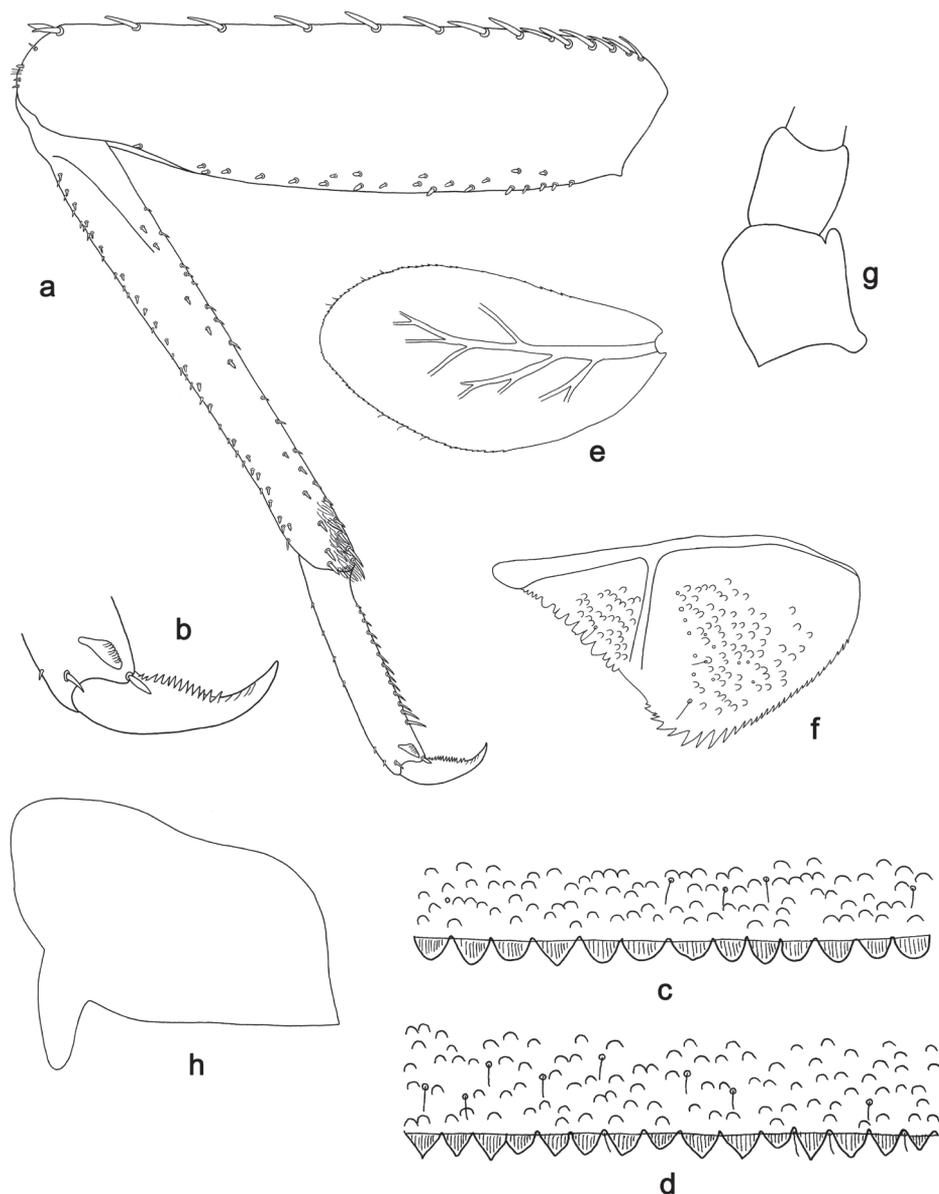
*Hypopharynx* (Fig. 5f). Lingua approx. as long as superlingua. Lingua longer than broad; medial tuft of stout setae poorly developed; distal half laterally expanded. Superlingua rounded; lateral margin rounded; fine, long, simple setae along distal margin.

*Maxilla* (Fig. 5g). Galea-lacinia with two simple, robust apical seta under crown. Inner dorsal row of setae with three denti-setae, distal denti-seta tooth-like, middle and proximal denti-setae slender, bifid and pectinate. Medially with one bipectinate, spine-like seta and four medium, simple setae. Maxillary palp 1.2× as long as length of galea-lacinia; 2-segmented; palp segment II 1.6× length of segment I; setae on maxillary palp fine and simple, scattered over surface of segments I and II; apex of last segment rounded, with excavation at inner distolateral margin.

*Labium* (Fig. 5h, i). Glossa basally broad, narrowing toward apex; shorter than paraglossa; inner margin with seven or eight spine-like setae increasing in length distally; apex with two long and one medium, robust, pectinate setae; outer margin with five or six long, spine-like setae; ventral surface with short, fine, simple, scattered setae. Paraglossa sub-rectangular, curved inward; apex rounded; with three rows of long, robust, distally pectinate setae in apical area and two medium, simple setae in anteromedial area; dorsally with a row of three long, spine-like setae near inner margin. Labial palp with segment I 0.9× length of segments II and III combined. Segment I ventrally with short, fine, simple setae. Segment II with large, lobed distomedial protuberance; distomedial protuberance 0.7× width of base of segment III; inner and outer margin with short, fine, simple setae; dorsally with two medium, spine-like, simple setae near outer margin. Segment III slightly pentagonal; apex truncate; length 1.1× width; ventrally covered with short, spine-like, simple setae and short, fine, simple setae.

*Hindwing pads* (Fig. 6h) well developed.

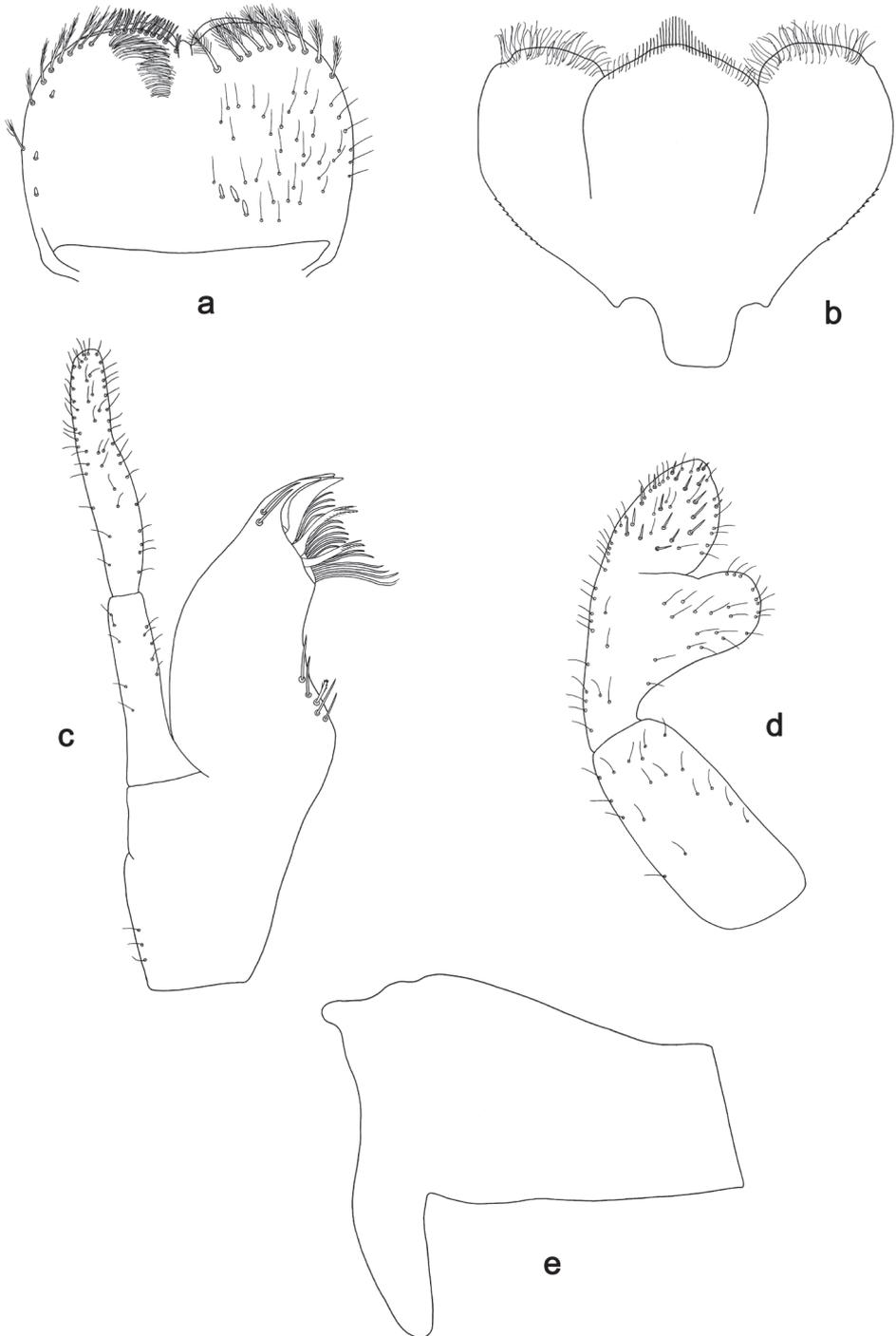
*Foreleg* (Fig. 6a, b). Ratio of foreleg segments 1.1:1.0:0.4:0.2. *Femur*. Length ca. 4× maximum width. Dorsal margin with a row of 12–14 curved, spine-like setae; length of setae 0.26× maximum width of femur. Apex rounded, with one pair of curved, spine-like setae and some short, stout setae. Many short, stout, lanceolate setae scattered along the ventral margin; femoral patch absent. *Tibia*. Dorsal margin with a row of short, stout setae, on apex one longer seta, and a row of short, stout setae close to dorsal margin. Ventral margin with a row of curved, spine-like setae, on apex two spine-like seta and a tuft of long, fine, simple setae. Anterior surface scattered with stout, lanceolate setae. Patellotibial suture present on basal 1/3 area. *Tarsus*. Dorsal



**Figure 6.** *Labiobaetis dayakorum* sp. nov., larva morphology: **a** Foreleg **b** Fore claw **c, d** Tergum IV **e** Gill IV **f** Paraproct **g** Antennal scape **h** Metanotum.

margin with a row of short, stout setae. Ventral margin with a row of curved, spine-like setae. Tarsal claw with one row of 9–13 denticles; distally pointed; with four stripes; subapical setae absent.

*Terga* (Fig. 6c, d). Surface with irregular rows of U-shaped scale bases and scattered fine, simple setae. Posterior margin of tergum IV with rounded or triangular spines, wider than long.



**Figure 7.** *Labiobaetis paraerosus*, larva morphology: **a** Labrum **b** Hypopharynx **c** Maxilla **d** Labial palp **e** Metanotum.

*Gills* (Fig. 6e). Present on segments I - VII. Margin with small denticles intercalating fine simple setae. Tracheae extending from main trunk to inner and outer margins. Gill I as long as length of 1/2 segment II. Gill IV as long as length of segments V and 1/3 VI combined. Gill VII as long as length of segments VIII and 1/3 IX combined.

*Paraproct* (Fig. 6f). Distally not expanded with 30–37 stout marginal spines. Surface scattered with U-shaped scale bases, fine, simple setae and micropores. Cercotractor with medium marginal spines.

**Etymology.** Dedicated to the indigenous Dayak people of Borneo.

**Distribution.** Indonesia: Kalimantan (Fig. 15c).

**Biological aspects.** The specimens were collected at an altitude of 200 m, partly in a large river.

**Type-material. Holotype.** Larva (on slide, GBIFCH 00592281), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan, tributary, 03°00.08'N, 116°30.80'E, 28.III.2001, P. Derleth and B. Feldmeyer leg. **Paratypes.** 1 larva (on slide, GBIFCH 00592255), Indonesia, East Kalimantan, Bas. Malinau, River Rian, loc. Seturan (1998-block 32-33), tributary, 03°00.95'N, 116°32.27'E, 30.III.2001, P. Derleth leg.; 1 larva (on slide, GBIFCH 00592256), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (2001-block 57), trib. Benganau, 02°59.37'N, 116°30.77'E, 11.IV.2001, P. Derleth and B. Feldmeyer leg. All material deposited in MZL.

### Not assigned to a group

#### *Labiobaetis borneensis* (Müller-Liebenau, 1984)

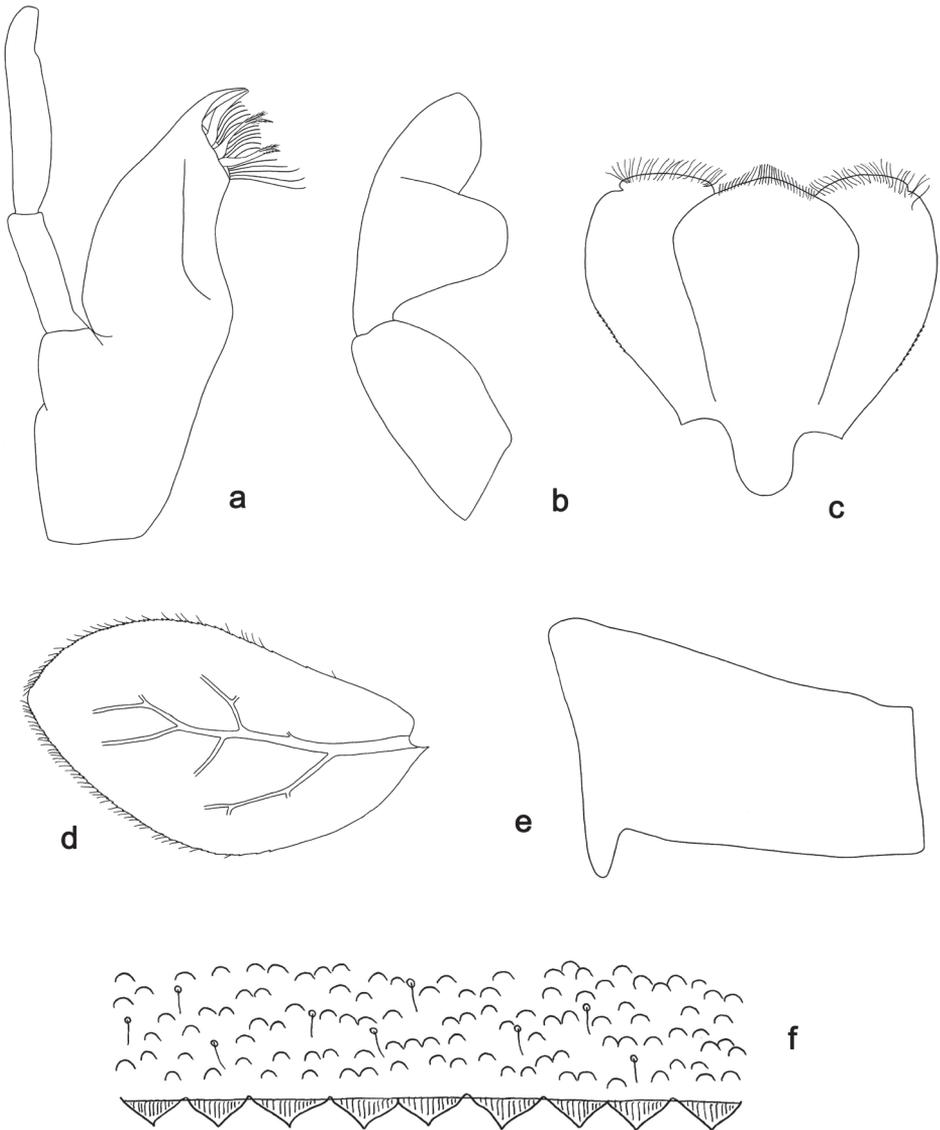
Figures 8, 10c, 12b, 13b, 15b

**Diagnosis. Larva.** Following combination of characters: A) dorsal surface of labrum with submarginal arc of 9–10 feathered setae (Müller-Liebenau 1984b: fig. 2a); B) labial palp segment II with a large, lobed distomedial protuberance, segment III oblong, apically slightly pointed; C) fore femur rather slender, length 3.6× maximum width, dorsal margin with a row of 11–13 curved, spine-like setae (Müller-Liebenau 1984b: fig. 2i); D) seven pairs of gills; E) hindwing pads present, small; F) distolateral process at scape well developed (Müller-Liebenau 1984b: fig. 2f).

**Description. Male imago** (Fig. 12b, 13b). Body length 4.6 mm, forewing length 4.5 mm.

**Colouration.** Head beige. Turbinate eyes dark orange brown, shaft slightly lighter. Thorax beige, pronotum dark olive brown, mesonotum olive. Wings hyaline, venation hyaline. Abdomen: terga olive, sterna transparent.

**Forewing** (Fig. 12b). Pterostigma with seven cross-veins, only two proximal ones reaching subcostal vein; double intercalary veins shorter than distance between corresponding main veins at wing margin.



**Figure 8.** *Labiobaetis borneoensis*, larva morphology: **a** Maxilla **b** Labial palp **c** Hypopharynx **d** Gill IV **e** Metanotum **f** Tergum IV.

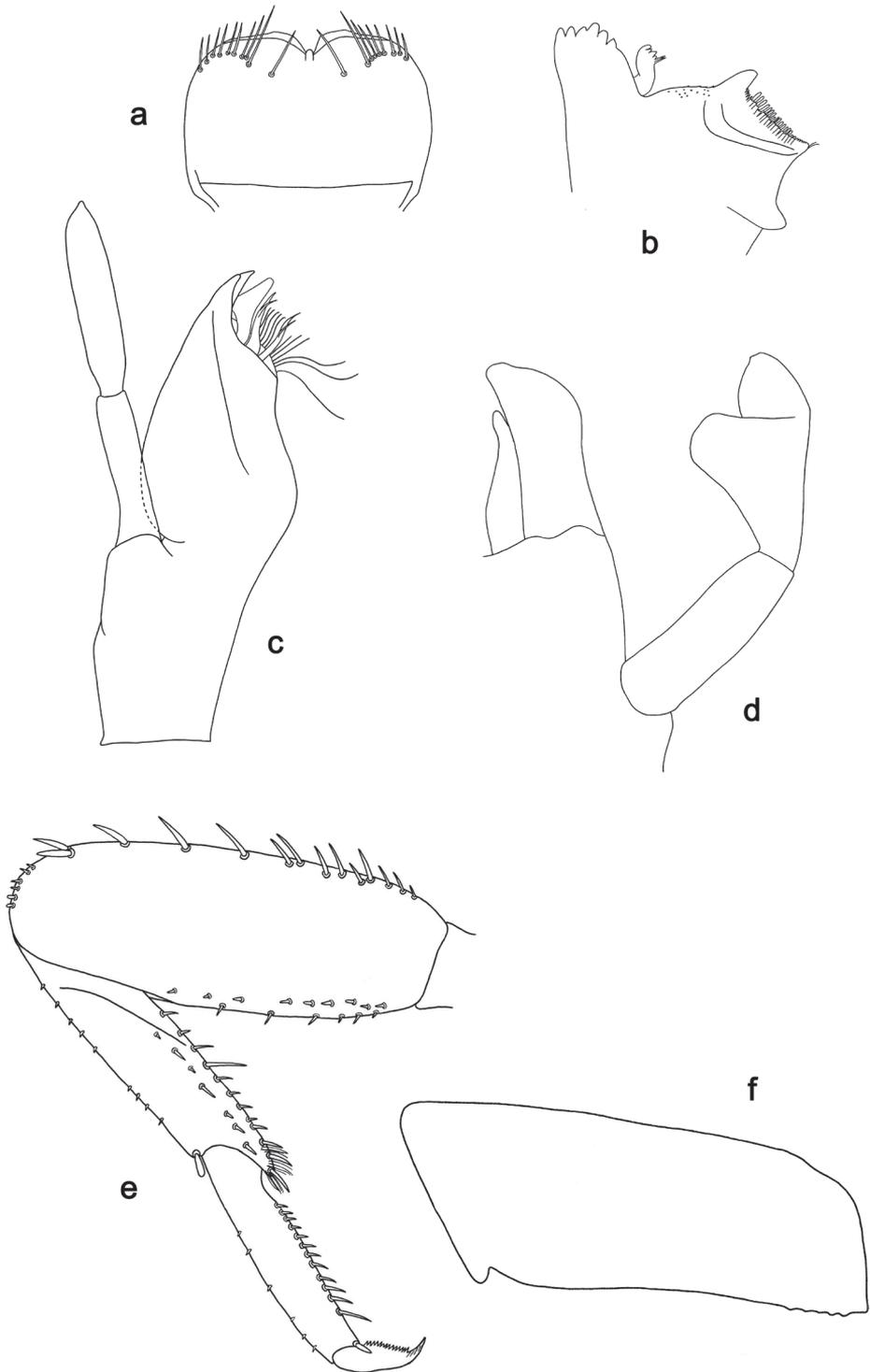
**Genitalia** (Fig. 13b). Basal segment of gonostylus (unistyler) with inner margin apically slightly expanded; segments I and II almost completely fused; constriction at base of segment II; segment III quadrangular. Styler plate between unistyligers trapezoidal, distal margin slightly concave.

**Distribution.** Indonesia: Kalimantan, Malaysia: Sabah, Brunei (Fig. 15b).

**Biological aspects.** The specimens were collected at altitudes between 100 m to 300 m, partly on bottom gravel, rock surface or submerged wood in stream run or riffles.

**Ontogenetic association.** With genetics, one male imago shares an identical COI sequence with a larva from the same location (K2P 0%, Table 3).

**Examined material.** 11 larvae (2 on slides, GBIFCH 00592240, GBIFCH 00658085, 9 in alcohol, GBIFCH 00515368, GBIFCH 00515370), Brunei, Temburong District, Ulu Temburong National Park, Belalong River (near field station), 04°32.82'N, 115°09.50'E, 100 m, V. 2014, K. Baker leg.; 1 larva (in alcohol, GBIFCH 00515369), Brunei, Temburong District, Ulu Temburong National Park, Sungai Seluju (small tributary to Temburong River, near station), 04°33.83'N, 115°08.92'E, 90 m, V. 2014, K. Baker leg.; 3 larvae (2 on slides, GBIFCH 00658081, GBIFCH 00592244, 1 in alcohol, GBIFCH 00515372), Malaysia, Sabah, Tawau River, primary forest, 04°24.08'N, 117°53.35'E, 280 m, 12.III.2008, Mendoza leg.; 1 male imago (in alcohol and wing on slide, GBIFCH 00672289, GBIFCH 00606854), Malaysia, Sabah, Tawau River, primary forest, 04°24.08'N, 117°53.35'E, 280 m, 12.III.2008, Mendoza leg.; 9 larvae (1 on slide, GBIFCH00465236, 8 in alcohol, GBIFCH 00515394, GBIFCH 00515309, GBIFCH 00515296, GBIFCH 00515376, GBIFCH 00515299), Indonesia, East Kalimantan, Bas. Malinau, River Rian, loc. Langap South (1997-bloc 6), trib. Belakau, 03°04.07'N, 116°30.43'E, 07.VII.2000, P. Derleth leg.; 14 larvae (in alcohol, GBIFCH 00515392, GBIFCH 00515393, GBIFCH 515315, GBIFCH 515312, GBIFCH 515306), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (2001-bloc 57), trib. Bengahau, 02°59.37'N, 116°30.77'E, 08.VIII.2000, P. Derleth leg.; 6 larvae (in alcohol, GBIFCH 00515317, GBIFCH 00515321, GBIFCH 00515383), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (1999-block 39-40), trib. Temalat (Sungai Guang), 03°00.17'N, 116°32.40'E, 01.VII.2000, P. Derleth leg.; 1 larva (in alcohol, GBIFCH 00515313), Indonesia, East Kalimantan, Bas. Malinau, River Rian, loc. Langap South (1995), trib. Ngayo, 03°01.80'N, 116°29.80'E, 08.VII.2000, P. Derleth leg.; 2 larvae (in alcohol, GBIFCH 00515382, GBIFCH 00515310), Indonesia, East Kalimantan, Bas. Malinau, River Rian, Langap South (1995), trib. Ngayo, 03°04.93'N, 116°30.97'E, 13.VII.2000, P. Derleth leg.; 3 larvae (in alcohol, GBIFCH 00515395, GBIFCH 00515297, GBIFCH 00515378), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (2000-block 43), trib. Temalat (Sungai Guang), 02°59.48'N, 116°33.48'E, 16.VIII.2000, P. Derleth and R. Schlaepfer leg.; 3 larvae (1 on slide, GBIFCH00465237, 2 in alcohol, GBIFCH 00515307, GBIFCH 00515375), Indonesia, East Kalimantan, Bas. Malinau, River Rian, loc. Seturan (1998-block 32-33), tributary, 03°00.95'N, 116°32.27'E, 30.III.2001, P. Derleth leg.; 1 larva (in alcohol, GBIFCH 00515316), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan (1999-block 27), tributary, 03°00.95'N, 116°30.52'E, 10.VII.2000, P. Derleth leg.; 4 larvae (in alcohol, GBIFCH 00515322, GBIFCH 00515377, GBIFCH 515379), Indonesia, East Kalimantan, Bas. Malinau, River Rian, loc. Langap South (1999-block 24), tributary, 03°01.67'N, 116°31.08'E, 11.VII.2000, P. Derleth leg.; 3 larvae (in alcohol, GBIFCH 00515380, GBIFCH 00515381), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan, main river, 03°00.08'N, 116°30.80'E, 28.III.2001, P. Derleth and B. Feldmeyer leg.; 5 larvae (in alcohol, GBIFCH 00515374), Indonesia, East Kalimantan, Bas. Malinau, River Seturan, loc. Seturan, tributary, 02°59.82'N, 116°31.37'E, 27.IV.2001, P. Derleth and M. Sartori leg. All material deposited in MZL.



**Figure 9.** *Labiobaetis moriharai*, larva morphology: **a** Labrum **b** Left mandible **c** Maxilla **d** Labium **e** Foreleg **f** Metanotum.

***Labiobaetis moriharai* (Müller-Liebenau, 1984)**

Figures 9, 11c, 15a

**Diagnosis. Larva.** Following combination of characters: A) dorsal surface of labrum with submarginal arc of 1 + 8–10 simple setae, the first three after central seta longer than others and decreasing in length; B) labial palp segment II with a large, lobed distomedial protuberance, segment III conical, apically slightly truncate; C) fore femur rather broad, length 3.4× maximum width, dorsal margin with a row of ca. 10 curved, spine-like setae; D) six pairs of gills; E) hindwing pads present, minute; F) scape with well-developed distolateral process (Müller-Liebenau 1984a: fig. 10f); G) paraproct distally not expanded, with ca. 12 stout marginal spines (Müller-Liebenau 1984a: fig. 10l).

**Distribution.** Malaysia: Selangor, Sabah; Vietnam; Brunei (Fig. 15a).

**Biological aspects.** The specimens were collected at altitudes from 100 m to 300 m, partly on bottom gravel, rock surface or vegetation in stream run or riffles.

**Examined material. Paratype.** 1 larva (on slide, no. 41), W. Malaysia, Trib. of Gombak River, 16 ½ miles N of Kuala Lumpur, 14.XI.[19]68, Coll. Bishop. **Other material.** 1 larva (on slide, GBIFCH 00658106), Brunei, Temburong District, Ulu Temburong National Park, Belalong River (near field station), 04°32.82'N, 115°09.50'E, 100 m, V. 2014, K. Baker leg.; 1 larva (on slide, GBIFCH 00592243), Brunei, Temburong District, Ulu Temborong National Park, Belalong River tributary, 04°32.63'N, 115°08.85'E, 170 m, V. 2014, K. Baker leg.; 5 larvae (2 on slides, GBIFCH 00592241, GBIFCH 00658112, 3 in alcohol, GBIFCH 00515325), Malaysia, Sabah, Tawau River, primary forest, 04°24.23'N, 117°53.58'E, 280 m, 12.III.2008, Mendoza leg. All material deposited in MZL, except paratype in Zoologische Staatssammlung München (ZSM).

**Key to the *Labiobaetis* species of Borneo (larvae)**

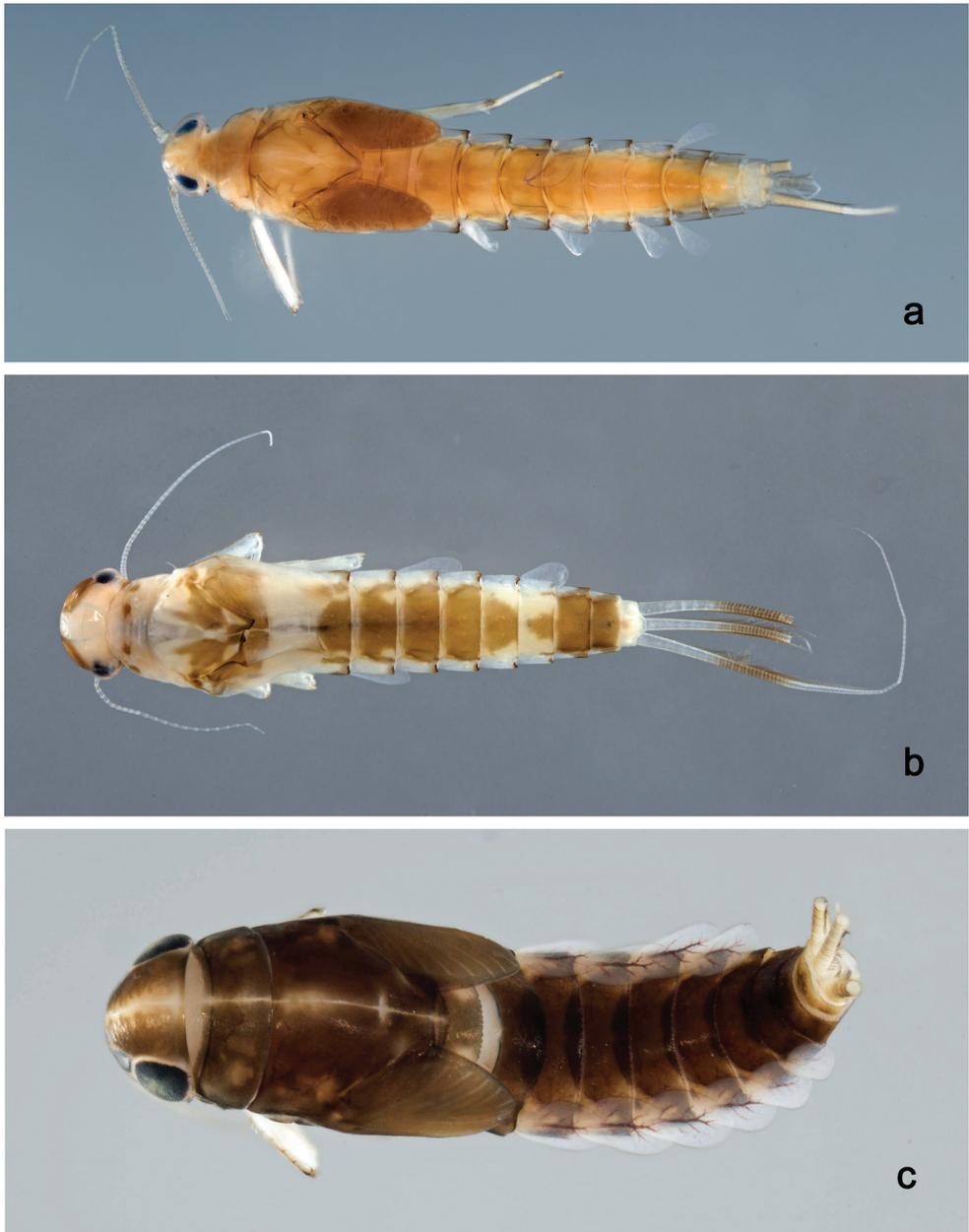
- 1 Dorsal surface of labrum with submarginal arc of clavate setae; hindwing pads absent ..... **2**
- Dorsal surface of labrum with submarginal arc of simple or feathered setae; hindwing pads present ..... **3**
- 2 Dorsal surface of labrum with submarginal arc of 13–15 setae; 8–11 setae on dorsal margin of femur; gills margin serrated with small denticles and with medium fine, simple setae ..... ***L. bakerae* sp. nov.**
- Dorsal surface of labrum with submarginal arc of 18–22 setae; 15–19 setae on dorsal margin of femur; gills margin serrated with small denticles and with both short and medium, fine, simple setae ..... ***L. penan* sp. nov.**
- 3 Dorsal surface of labrum with submarginal arc of simple setae; hindwing pads minute (Fig. 9f) ..... ***L. moriharai***
- Dorsal surface of labrum with submarginal arc of feathered setae ..... **4**
- 4 Hindwing pads small (Fig. 8e) ..... ***L. borneoensis***
- Hindwing pads well developed (Fig. 6h) ..... ***L. dayakorum* sp. nov.**



**Figure 10.** Habitus, larvae, dorsal view: **a** *Labiobaetis bakerae* sp. nov. **b** *Labiobaetis penan* sp. nov. **c** *Labiobaetis borneoensis*.

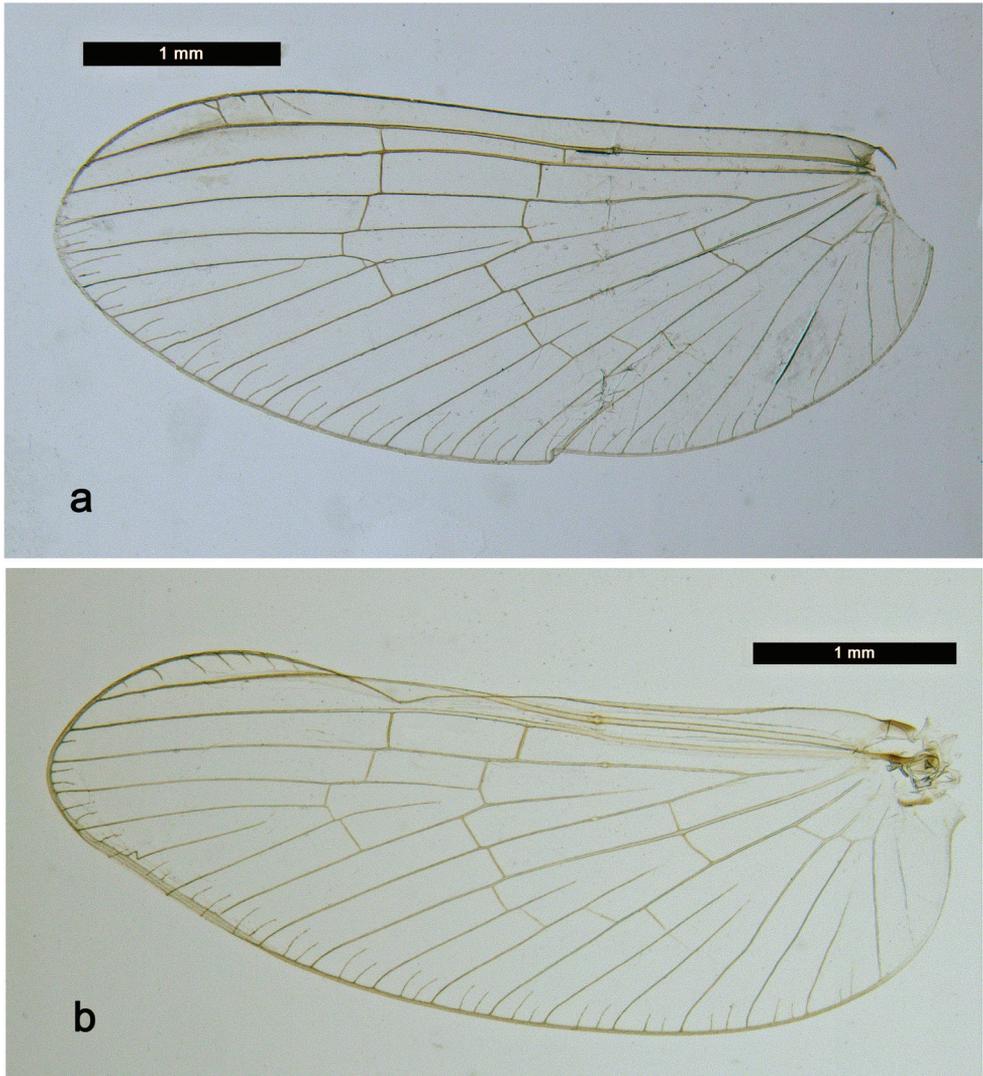
### Distribution

The material treated in this study was collected in ca. 20 localities in Borneo, which belong to four different areas, one area in Brunei, two in Sabah (Malaysia), and one in Kalimantan (Indonesia) (Fig. 15). There are still many regions in Borneo as well



**Figure 11.** Habitus, larvae, dorsal view: **a** *Labiobaetis dayakorum* sp. nov. **b** *Labiobaetis paraoperosus* **c** *Labiobaetis moriharai*.

as in Southeast Asia in general where no sampling of mayflies has yet been done and many species known to date are from a single population only. This implies that the diversity and the distribution must be considered as very preliminary. However, the distribution of the *Labiobaetis* species seems to be very diverse. *Labiobaetis moriharai*



**Figure 12.** Male imagos, forewings: **a** *Labiobaetis penan* sp. nov. **b** *Labiobaetis borneoensis*.

has a large distribution (continental and insular) and the other species are endemic to Borneo (Fig. 15). In terms of altitude, the *Labiobaetis* species of Borneo were found from sea level to mountain areas up to 1,450 m. The GPS coordinates of the locations of examined material are given in Table 2.

### Genetics

COI sequences were obtained from two of the three new species (Table 1) as well as from the two other species. In two cases (*L. penan* sp. nov. and *L. borneoensis*) a male

**Table 2.** GPS coordinates of locations of examined specimens.

Species	Locality	GPS coordinates	
<i>L. bakerae</i> sp. nov.	Brunei	04°32.77'N, 115°09.52'E	
		04°32.92'N, 115°09.45'E	
		04°33.07'N, 115°09.41'E	
<i>L. penan</i> sp. nov.	Indonesia: Kalimantan	03°01.67'N, 116°31.08'E	
		03°04.07'N, 116°30.43'E	
		03°00.17'N, 116°32.40'E	
		03°04.93'N, 116°30.97'E	
		02°59.37'N, 116°30.77'E	
		03°00.15'N, 116°32.42'E	
		02°59.20'N, 116°33.18'E	
		02°59.48'N, 116°33.48'E	
		03°01.80'N, 116°29.80'E	
		03°00.95'N, 116°32.27'E	
		03°00.95'N, 116°30.52'E	
		Brunei	04°32.82'N, 115°09.50'E
			04°32.83'N, 115°09.38'E
04°32.63'N, 115°08.85'E			
<i>L. dayakorun</i> sp. nov.	Malaysia: Sabah	06°00.40'N, 116°32.80'E	
	Indonesia: Kalimantan	03°00.08'N, 116°30.80'E	
		03°00.95'N, 116°32.27'E	
<i>L. borneoensis</i> (Müller-Liebenau)	Indonesia: Kalimantan	02°59.37'N, 116°30.77'E	
		03°04.07'N, 116°30.43'E	
		02°59.48'N, 116°33.48'E	
		02°59.37'N, 116°30.77'E	
		03°00.17'N, 116°32.40'E	
		03°01.80'N, 116°29.80'E	
		03°04.93'N, 116°30.97'E	
		03°00.95'N, 116°32.27'E	
		03°01.67'N, 116°31.08'E	
		03°00.08'N, 116°30.80'E	
		02°59.82'N, 116°31.37'E	
		Brunei	04°32.82'N, 115°09.50'E
		04°33.83'N, 115°08.92'E	
Malaysia: Sabah	04°24.08'N, 117°53.35'E		
<i>L. moriharai</i> (Müller-Liebenau)	Malaysia: Selangor	03°13.07'N, 101°42.75'E	
	Malaysia: Sabah	04°24.23'N, 117°53.58'E	
	Brunei	04°32.82'N, 115°09.50'E	
		04°32.63'N, 115°08.85'E	

imago could be associated with larvae: the COI sequences of the two ontogenetic stages were identical. The genetic distances (K2P) between the species in Borneo are between 19% and 25%, and therefore much higher than 3.5%, which is generally considered as a likely maximal value for intraspecific divergence (Hebert et al. 2003, Ball et al. 2005, Zhou et al. 2010) (Table 3). Very limited genetic distances (between 0%

**Table 3.** Genetic distances (COI) between sequenced specimens, using the Kimura 2-parameter.

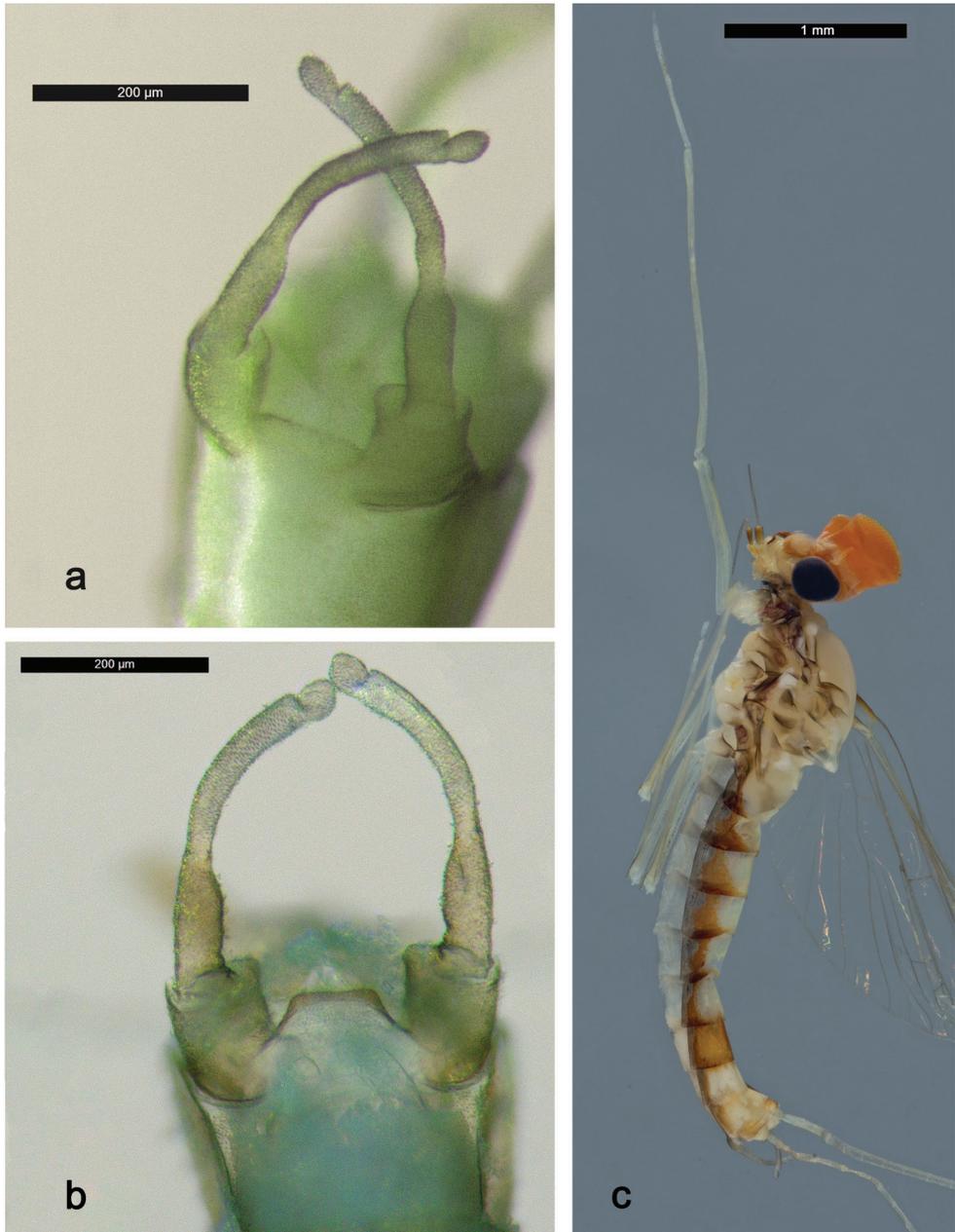
		1	2	3	4	5	6	7	8
1	<i>L. bakerae</i> sp. nov.	larva							
2	<i>L. bakerae</i> sp. nov.	larva	0.06						
3	<i>L. bakerae</i> sp. nov.	larva	0.06	0.01					
4	<i>L. penan</i> sp. nov.	larva	0.22	0.20	0.20				
5	<i>L. penan</i> sp. nov.	larva	0.22	0.20	0.20	0.00			
6	<i>L. penan</i> sp. nov.	imago	0.22	0.20	0.20	0.00	0.00		
7	<i>L. borneoensis</i> (Müller-Liebenau)	larva	0.20	0.19	0.19	0.21	0.21	0.21	
8	<i>L. borneoensis</i> (Müller-Liebenau)	imago	0.20	0.19	0.19	0.21	0.21	0.21	0.00
9	<i>L. moribarai</i> (Müller-Liebenau)	larva	0.25	0.22	0.23	0.22	0.22	0.20	0.20

and 1%) were found between specimens of the same species, as in *L. penan* sp. nov., *L. borneoensis*, and partly in *L. bakerae* sp. nov. The only exception is *L. bakerae* sp. nov.: in this species one larva has a distance of 6% from the two others, despite being collected in the same area and having no morphological difference.

## Discussion

For the assignment of the new species to *Labiobaetis* we are referring to Kluge and Novikova (2014), Müller-Liebenau (1984a) and McCafferty and Waltz (1995). *Labiobaetis* is characterized by a number of derived characters, some of which are not found in other taxa (Kluge and Novikova 2014): antennal scape sometimes with a distolateral process (Fig. 6g); maxillary palp two segmented with excavation at inner distolateral margin of segment II, excavation may be poorly developed or absent (Kaltenbach and Gattolliat 2019: figs 1o–q); labium with paraglossae widened and glossae diminished; labial palp segment II with distomedial protuberance (Kaltenbach and Gattolliat 2019: fig. 1g–n). All these characters vary and may be secondarily lost (Kluge and Novikova 2014). The concept of *Labiobaetis* is also based on additional characters, summarized and discussed in Kaltenbach and Gattolliat (2018, 2019).

Two of the three new species (*L. bakerae* sp. nov., *L. penan* sp. nov.) belong to the rather large *sumigarensis* group and the third one (*L. dayakorum* sp. nov.) to the *operosus* group (Müller-Liebenau and Hubbard 1985, Kaltenbach and Gattolliat 2019). *Labiobaetis bakerae* sp. nov. and *L. penan* sp. nov. can be distinguished by the number of clavate setae forming an arc on the dorsal surface of the labrum (13–15 in *L. bakerae* sp. nov., ca. 22 in *L. penan* sp. nov.), the number of setae at the dorsal margin of the femur (8–11 in *L. bakerae* sp. nov., 15–19 in *L. penan* sp. nov.) and the presence of split tips of the marginal spines of the paraproct in *L. penan* sp. nov. (Fig. 4h). *Labiobaetis bakerae* sp. nov. is morphologically closely related to *L. jacobusi* Kubendran and Balasubramanian from India and *L. geminatus* (Müller-Liebenau and Hubbard) from Sri Lanka (Müller-Liebenau and Hubbard 1985, Kubendran et al. 2015). From the first species *L. bakerae* sp. nov. is different in the shape of the labial palp, the longer maxil-



**Figure 13.** Male imagos: **a** *Labiobaetis penan* sp. nov., genitalia **b** *Labiobaetis borneoensis*, genitalia **c** *Labiobaetis penan* sp. nov., imago, lateral view.

lary palp (compared to galea-lacinia) and the shorter medial tuft of the hypopharynx (Fig. 1f–h; Kubendran et al. 2015: figs 44, 47, 48). From the second species, *L. bakerae* sp. nov. differs by the very poorly developed distolateral scape process (rather well developed in *L. geminatus*), the shape of the labial palp (distomedial protuberance of seg-



**Figure 14.** Larval habitats: **a, b** *Labiobaetis bakerae* sp. nov., photos Kate Baker.

ment II more slender and with a clearly concave distal outer margin in *L. geminatus*), the distinct denticles between prostheca and mola of the left mandible (hardly visible in *L. geminatus*), the maxillary palp with a pronounced distolateral excavation (less developed in *L. geminatus*) and the shape of the triangular spines at anterior margin of tergum IV (generally much wider than long; as wide as long in *L. geminatus*, with

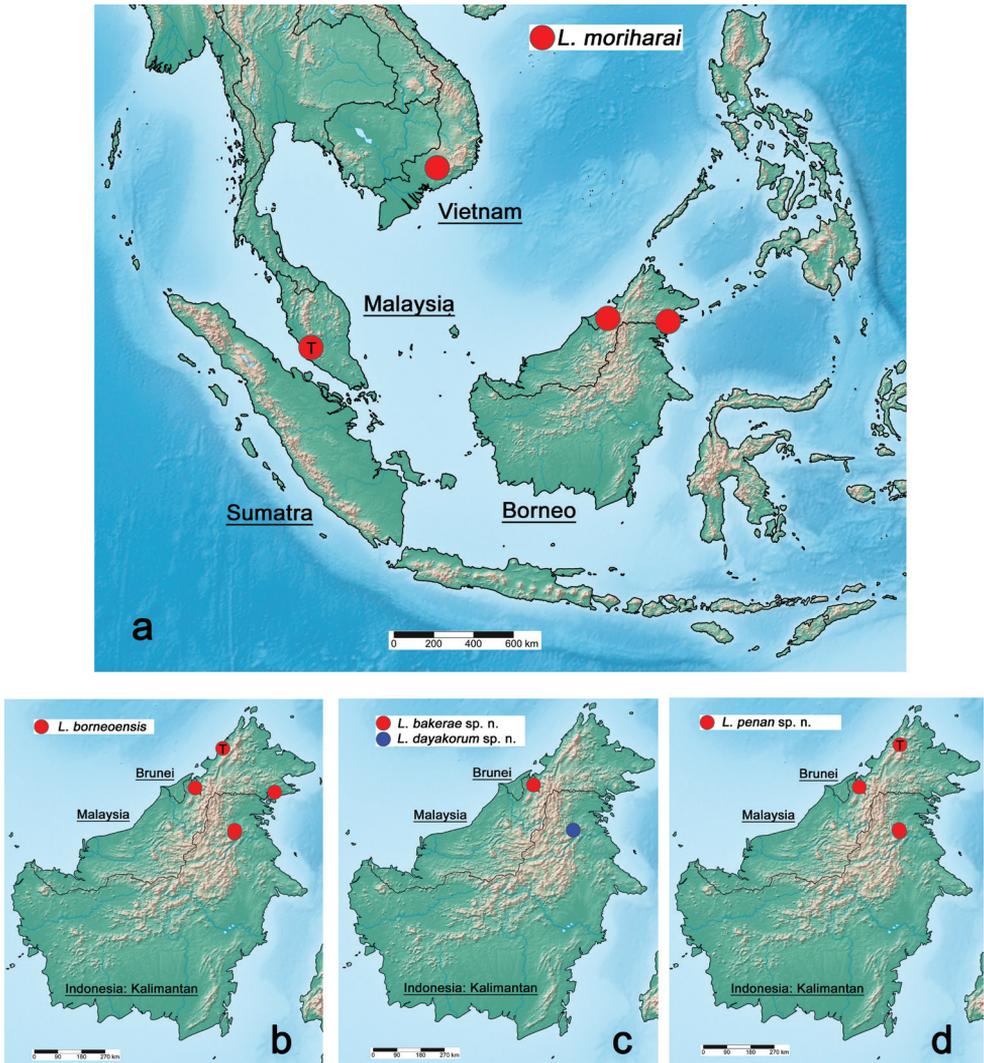
pronounced points) (Figs 1b, g, h, 2d, g; Müller-Liebenau and Hubbard 1985: figs 5b, d, e, g, 22). The third new species, *L. dayakorum* sp. nov., is morphologically close to *L. paraoperosus* Kaltenbach and Gattolliat from Sumatra, but differentiated in the following characters: thorax and abdomen of *L. dayakorum* sp. nov. dorsally uniform brown (Fig. 11a) and with a distinct pattern in *L. paraoperosus* (Fig. 11b), shape of the labial palp (Figs 5h, 7d), denticles of the right mandible (4+1+3 in *L. dayakorum* sp. nov., 4+3 in *L. paraoperosus*), and size and shape of the hindwing pads (Figs 6h, 7e).

In general, the genetic distances between the different species of *Labiobaetis* are rather high in Borneo, between 19% and 25% (K2P, Table 3), which is in line with the genetic distances found in New Guinea (avg. 22%; Kaltenbach and Gattolliat 2018) and Indonesia (11%-24%; Kaltenbach and Gattolliat 2019). Ball et al. (2005) reported a mean interspecific, congeneric distance of 18% for mayflies from the United States and Canada.

The intraspecific distances are mostly very low as expected, ranging from 0% to 1% (K2P). This result is certainly biased as it is based on a limited number of sequenced specimens per species, which were mostly from a single population. But there is one exception, *L. bakerae* sp. nov., where one specimen has an intraspecific distance of 6% to another specimen of the same population as well as to a specimen of another population. Compared to the usual distances between different *Labiobaetis* species in that region and because there is no morphological difference, this distance is surprising, but can be still considered as intraspecific. Ball et al. (2005) also reported a case with 6% intraspecific distance in a mayfly in North America and intraspecific K2P distances of more than 3.5% are also not uncommon within Plecoptera (Gill et al. 2015, Gattolliat et al. 2016).

In addition to the five species cited in this paper, we obtained two additional COI sequences with clearly interspecific genetic distance to other specimens with similar morphology. In one case, one specimen is highly similar to *L. borneoensis*, but with a K2P distance of 16%. In the other case, one specimen is morphologically very close to *L. penan* sp. nov. and partly damaged, but with a K2P distance of 22%. Because of the limited amount of material and the absence of morphological support, they have to remain species hypotheses for now without further treatment in this paper. Additional material will be necessary to confirm their status in the future. We also have specimens of two additional undescribed species, which have some morphological differences to their closest species. Unfortunately, the material is insufficient or partly damaged and we could not extract DNA. We therefore also refrain to describe them.

The number of sampled localities and different habitats is still very limited and there are large regions, especially in mountainous areas, without any collection activities so far (Fig. 15). In addition, we have four species hypotheses based on genetics only or based on morphological differences without genetics, which may be confirmed as valid species in the future. Therefore, we may assume that the number of *Labiobaetis* species in Borneo will continue to increase substantially with further collections in the future. Thereby, inter-disciplinary collaborations between ecologists and taxonomists may contribute to the discovery of new species in these remote, tropical regions (Baker et al. 2019).



**Figure 15.** Distribution of *Labiobaetis* in Borneo: **a** *Labiobaetis moriharai* **b** *Labiobaetis borneoensis* **c** *Labiobaetis bakerae* sp. nov. and *Labiobaetis dayakorom* sp. nov. **d** *Labiobaetis penan* sp. nov. T: type locality.

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# Nomenclatural changes in *Centroptella* Braasch & Soldán, 1980 (Ephemeroptera, Baetidae)

Nikita J. Kluge<sup>1</sup>, Roman J. Godunko<sup>2,3</sup>, Marek Svitok<sup>4,5</sup>

**1** Department of Entomology, Saint Petersburg State University, Universitetskaya nab., 7/9, Saint Petersburg, 199034, Russia **2** Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Branišovská 31, CZ-37005 České Budějovice, Czech Republic **3** Department of Invertebrate Zoology and Hydrobiology, University of Łódź, Banacha 12/16, 90237 Łódź, Poland **4** Faculty of Ecology and Environmental Sciences, Technical University in Zvolen, T. G. Masaryka 24, SK-96001 Zvolen, Slovakia **5** Department of Ecosystem Biology, Faculty of Science, University of South Bohemia, Branišovská 1760, CZ-37005, České Budějovice, Czech Republic

Corresponding author: Nikita J. Kluge (n.kluge@spbu.ru)

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## Abstract

The genus *Centroptella* Braasch & Soldán, 1980 is accepted here in a wide sense, i.e., including *Chopralla* Waltz & McCafferty, 1987. This genus concept is similar to the concept of the genus *Bungona* Harker, 1957 proposed by Salles et al. (2016), but with the generic name *Centroptella* instead of *Bungona*. The type species of *Bungona*, *B. narilla* Harker, 1957, has an unknown systematic position; the neotype designation proposed by Suter and Pearson (2001) is invalid, being inconsistent with the International Code of Zoological Nomenclature; the species name *B. narilla* and the generic name *Bungona* are *nomina dubia*, so the name *Centroptella* is the senior name for the genus under consideration. The generic names *Chopralla* and *Crassolus* Salles, Gattolliat & Sartori, 2016 both are junior synonyms of *Centroptella* (**syn. nov.**). The subgenera *Bungona*, *Centroptella* and *Chopralla* proposed by Salles et al. (2016) are unnatural. The following new combinations are proposed: *Centroptella bintang* (Marle, Salles & Gattolliat, 2016) **comb. nov.**, *Centroptella bifida* (Shi & Tong, 2019) **comb. nov.**, *Centroptella fusina* (Tong & Dudgeon, 2003) **comb. nov.**, *Centroptella fustipalpus* (Lugo-Ortiz & McCafferty, 1998) **comb. nov.**, *Centroptella illiesi* (Lugo-Ortiz & McCafferty, 1998) **comb. nov.**, *Centroptella inzingae* (Crass, 1947) **comb. nov.**, *Centroptella papilionodes* (Marle, Salles & Gattolliat, 2016) **comb. nov.**, *Centroptella pontica* (Sroka, Godunko & Gattolliat, in Sroka et al. 2019) **comb. nov.**, *Centroptella ovata* (Shi & Tong, 2019) **comb. nov.**, *Centroptella quadrata* (Shi & Tong, 2019) **comb. nov.** and *Centroptella saxophila* (Agnew, 1961) **comb. nov.** The two Australian

species, *C. fustipalpus* and *C. illiesi*, differ from each other in the shape of tergalii; corrections to the original description of *C. fustipalpus* are given based on re-examination of the holotype and paratypes; details of larval structures of *C. illiesi* are figured. Corrections to the former descriptions of the South African species *C. inzingae* and *C. saxophila* are given. Examination of type material led to the discovery that the original description of the Oriental species *Centroptella liebenauae* Soldán, Braasch & Muu, 1987 was based on two different species: the descriptions of imago and subimago belong to *Centroptella longisetosa* Braasch & Soldán, 1980 (the type species of *Centroptella*), and the description of larva belongs to a different species, which we describe here as *Centroptella ingridae* **sp. nov.** The holotype of *C. liebenauae*, a larva, should be considered lost; based on the date of collection, it belonged to *C. longisetosa*; a set of larval exuviae with the same collecting data as the holotype, is designated as the neotype of *C. liebenauae*, and a new synonymy is established: *C. longisetosa* = *C. liebenauae* **syn. nov.** The larvae originally assigned to *C. liebenauae* are placed to a new species *Centroptella ingridae* **sp. nov.** belonging to the *inzingae-ingridae* species group; all stages of development of this species are described based on male and female imagines reared from larvae in Thailand and on the misidentified paratypes of *C. liebenauae* from Vietnam. *Centroptella longisetosa* is redescribed based on the single paratype from China, the neotype and paratypes of *C. liebenauae* from Vietnam, and additional material from India. Additional data on the holotype of *Centroptella colorata* Soldán, Braasch & Muu, 1987 are given.

### Keywords

India, mayflies, new species, South Africa, systematics, Thailand, Vietnam

### Introduction

Initially, the genus *Centroptella* Braasch & Soldán, 1980 was established for a single species, *C. longisetosa* Braasch & Soldán, 1980 described from China. Subsequently, other species of *Centroptella* were described from the Oriental Region, i.e., *C. ceylonensis* Müller-Liebenau, 1983, *C. similis* Müller-Liebenau, 1983 and *C. soldani* Müller-Liebenau, 1983 from Sri Lanka, *C. pusilla* Müller-Liebenau, 1984 from Borneo, *C. liebenauae* Soldán, Braasch & Muu, 1987 and *C. colorata* Soldán, Braasch & Muu, 1987 from Vietnam. Waltz and McCafferty (1987a) synonymized *Centroptella* with *Cloeodes* Traver, 1938, and at the same time proposed a new genus *Chopralla* Waltz & McCafferty, 1987, so that *longisetosa* [*Centroptella*] and *soldani* [*Centroptella*] were placed by them in the genus *Cloeodes*, and *ceylonensis* [*Centroptella*], *similis* [*Centroptella*] and *pusilla* [*Centroptella*] were placed in the genus *Chopralla*. In accordance with this classification, *Cloeodes fustipalpus* Lugo-Ortiz & McCafferty, 1998 and *Cloeodes illiesi* Lugo-Ortiz & McCafferty, 1998 were described from Australia, and *Chopralla fusina* Tong & Dudgeon, 2003 was described from Hong Kong; however, these three species have all the characters of *Centroptella*. Suter and Pearson (2001) stated that the Australian species *fustipalpus* [*Cloeodes*] and *illiesi* [*Cloeodes*] were identical to *Bungona narilla* Harker, 1957 and, thus, belonged to the genus *Bungona* Harker, 1957. Salles et al. (2016) reasonably stated that the East Hemisphere taxa *Centroptella* and *Chopralla* are closely related and different from the West Hemisphere taxon *Cloeodes*. At the same time, they accepted the interpretation of *Bungona narilla* proposed by Suter and Pearson (2001), and based on this, moved all *Centroptella* and *Chopralla* to

the genus *Bungona* but treated these three taxa as subgenera. In accordance with this classification, *Bungona* (*Centroptella*) *papilionodes* Marle, Salles & Gattolliat, 2016 and *Bungona* (*Chopralla*) *bintang* Marle, Salles & Gattolliat, 2016 were described from Borneo; *Bungona* (*Chopralla*) *pontica* Sroka, Godunko & Gattolliat in Sroka et al. 2019 was described from Turkey; *Bungona* (*Centroptella*) *ovata* Shi & Tong, 2019, *Bungona* (*Centroptella*) *quadrata* Shi & Tong, 2019 and *Bungona* (*Chopralla*) *bifida* Shi & Tong, 2019 were described from China. Most descriptions were based on larvae only and, hence, lack some important taxonomic characters. Some of the species names mentioned above are synonyms, and some newly discovered (unpubl.) species of *Centroptella* from the Oriental and Afrotropical regions await description. Before these are described, however, the status of the formerly described taxa must be clarified.

## Material and methods

Imagines were reared from larvae in cages placed in natural flowing water and in containers with stagnant water. Part of material, including the holotype of *Centroptella ingridae* sp. nov., will be permanently deposited in the Zoological Institute of the Russian Academy of Sciences (Saint Petersburg, Russia) (**ZIN**), but is temporarily located in the Department of Entomology of Saint Petersburg State University. The type specimens of *C. longisetosa*, *C. liebenauae* and *C. colorata* reported in this paper, which are deposited in the Institute of Entomology, Biology Centre, **CAS** (České Budějovice, Czech Republic) were temporarily moved to the Department of Entomology of Saint Petersburg State University during this study. The type specimens of *C. fustipalpus* reported in this paper, are deposited in the Purdue University Entomological Research Collection (West Lafayette, Indiana, USA); slides for these specimens were made by L.M. Jacobus using Euparal or BioQuip slide-mounting media. Other slides were made using Canada Balsam. In order to examine internal parts of penis and genital muscles of fresh specimens, genitalia were kept in hot water to dissolve non-translucent white inclusions; for this purpose, a glass with water and separated genitalia was placed on the cover of a desk-lamp. In the lists of material examined, the following arbitrary abbreviations are used: **L** – larva; **S** – subimago; **I** – imago; **L-S-I**<sup>♂</sup> – male imago reared from larva, with larval and subimaginal exuviae; **L-S**<sup>♂</sup> – male subimago reared from larva, with larval exuviae; **L/S**<sup>♂</sup> – male subimago extracted from mature larva.

The term “microlepide” is used according to Kluge and Novikova (2014), the terms “gonovectis”, “unistyliger” and “sigilla” according to Kluge and Novikova (2011); the term “protopteron” according to Kluge (2005), other terms according to Kluge (2004). The noun “blank” is used to describe an unpigmented or pale area.

For scanning electron microscopy (Figs 110–122), samples were gradually transferred to acetone, critical point dried and coated with gold by sputtering using a Baltec SCD050 Sputter Coater. Observations were taken on the scanning electron microscope (SEM) Jeol JSM 7401F at 4 kV in the Laboratory of Electron Microscopy, Institute of Parasitology, Biology Centre, CAS (České Budějovice, Czech Republic).

Other samples (Figs 81–82 and 126–128) were dried taken directly from alcohol, coated with gold and observed on the scanning microscope Jeol JCM-5000 (Neoscope) at 15 kV in the Centre for Molecular and Cell Technologies of St. Petersburg State University.

## Results

### Status of the generic name *Bungona*

Originally, the genus *Bungona* was established for a single species, *Bungona narilla* Harker, 1957, which was described from Coal and Candle Creek, Ku-ring-gai Chase National Park, Sydney (Australia). This species description was based on one male imago (holotype), one female subimago and one larva. The reason these three specimens were placed in one species was not reported. The description contains evident errors (tarsi of middle and hind legs were regarded to be 5-segmented, gonostyli were regarded to be 4-segmented, paraprocts were confused with the penis); the combination of other characters is different from any known species. The holotype and paratypes of *B. narilla* were stated to be housed in the British Museum (Natural History) (Harker 1957: 63; Suter and Pearson 2001: 247), but they disappeared and have not been reported among type specimens of this museum (Kimmins 1971).

Dean and Suter (1996) and Suter (1997) determined larvae they collected in Australia (Queensland, New South Wales, Victoria and Tasmania) as belonging to *B. narilla*, and based on this, they redefined the genus *Bungona*. However, these larvae differ significantly from the original description of *B. narilla* by the following characters. The apical segment of the labial palp is ovoid, without any concavities and points (Suter and Pearson 2001: fig. 22; Webb and Suter 2010: fig. 7); in contrast to this, the original description states that “the distal segment truncate”, and it is figured with the apex sharply pointed and the free margin deeply concave (Harker 1957: fig. 56). Larvae reported by Dean and Suter have the prostheca of the right mandible located close to the canines, with the distal branch running along the canines and the proximal branch arising from it under the right or blunt angle (Suter and Pearson 2001: figs 14, 15; Webb and Suter 2010: fig. 4). In contrast, the original description of *B. narilla* features a right mandible figured with both branches of the prostheca directed proximally and diverging at an acute angle (Harker 1957: fig. 53). Such prostheca form is found in various non-related taxa of Baetidae, including some species of *Centroptella* (Fig. 41), but not in the Australian species which Dean and Suter determined as *B. narilla*.

Lugo-Ortiz and McCafferty (1998) described larvae of two Australian species of *Centroptella* under the names *Cloeodes fustipalpus* Lugo-Ortiz & McCafferty, 1998 and *Cloeodes illiesi* Lugo-Ortiz & McCafferty, 1998. Suter and Pearson (2001) synonymized both these species names with *Bungona narilla*. According to the original description, *C. fustipalpus* differs from *C. illiesi* by having a non-bifid right prostheca and widened tergalii. As for the first character, “The specimen they illustrated and described as *C. fustipalpus* had a broken prostheca (subsequently confirmed by Mc-

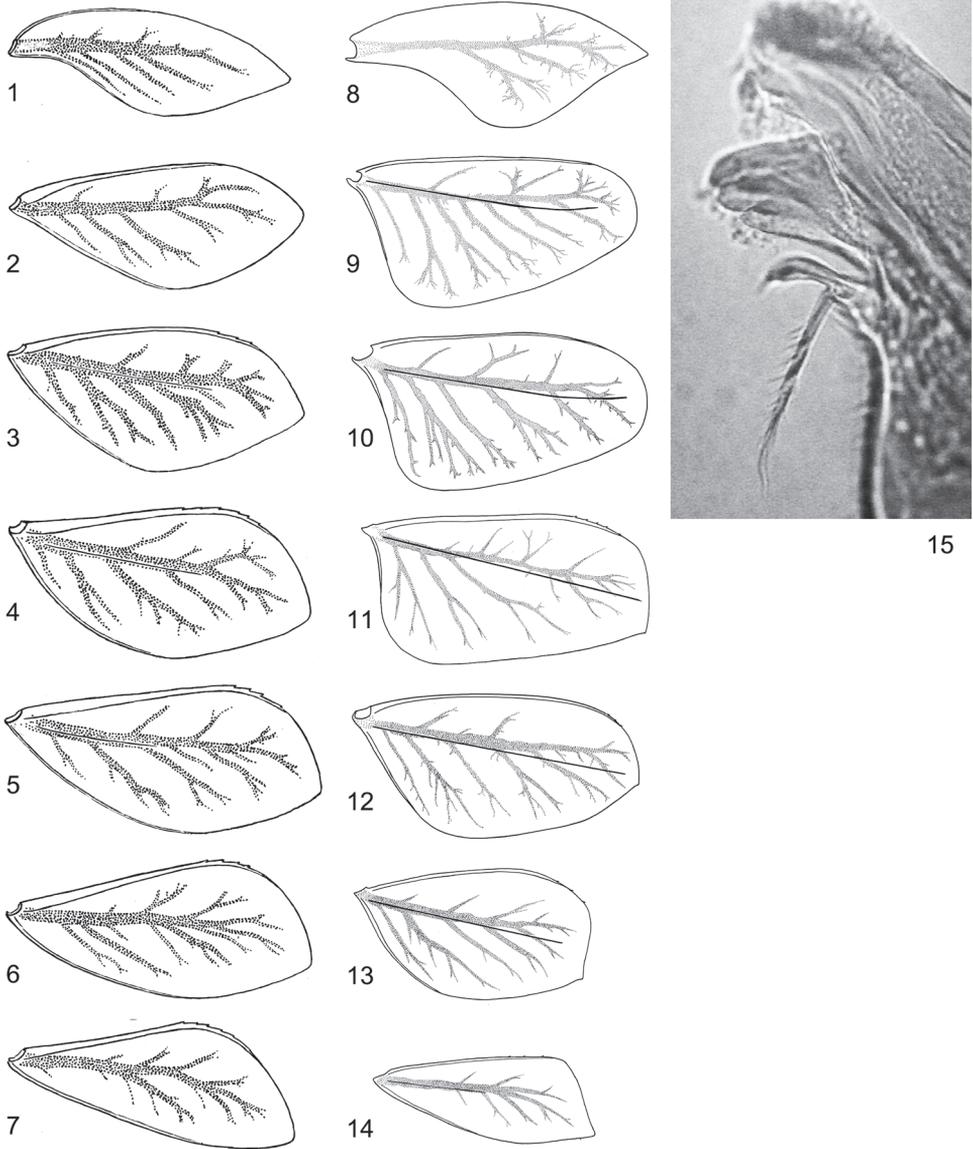
Cafferty, pers. comm.)” (Suter and Pearson 2001: 251). Our recent re-examination of the type material supports this conclusion: the proximal branch of the prosthema is not apparent on the right mandible of the paratype figured in the original description (Lugo-Ortiz and McCafferty 1998: fig. 3), but it is present on the holotype (Fig. 15). Concerning the shapes of tergalii, Suter and Pearson (2001) assumed that “this character may be influenced by age and environment”. However, descriptive data associated with various species of *Centroptella* from Asia and Africa (Kluge, unpublished) suggests that the shape of the tergalii is species-specific and constant at least among late larval instars. The holotype and all three paratypes of *C. fustipalpus* have tergalii of the 2<sup>nd</sup> and next pairs sharply widened proximally (Figs 8–14), which is quite different from the lanceolate tergalii of *C. illiesi* (Figs 1–7). The tergalii of the paratype of *C. fustipalpus*, which was figured in the original description as “Gill 4” (Lugo-Ortiz and McCafferty 1998: fig. 8) actually belongs to the 2<sup>nd</sup> pair (Fig. 9).

Suter and Pearson (2001) described male imagines ascribed to *B. narilla* based on specimens reared from larvae. In the same publication, they designated a neotype of *B. narilla*; this specimen is a non-reared male imago, collected from the Gara River, about 400 km north of the type locality. This choice of neotype (imago without associated larval exuviae) does not allow it to be compared with earlier described and reported forms, because most of them are known as larvae only. This neotype designation contradicts paragraphs 75.3.1, 75.3.5 and 75.3.6 of the International Code of Zoological Nomenclature (ICZN) and is invalid for the following three reasons.

1) “A neotype is validly designated when there is an exceptional need and only when that need is stated expressly and when the designation is published with the following particulars: 75.3.1. a statement that it is designated with the express purpose of clarifying the taxonomic status or the type locality of a nominal taxon ...”. All species taken into account in the publication, where the neotype of *B. narilla* was designated, i.e., *narilla* [*Bungona*], *fustipalpus* [*Cloeodes*] and *illiesi* [*Cloeodes*], were regarded as belonging to one species, and all their characters hitherto regarded as species-specific, were regarded as individual variability. In this situation, neotype designation is unnecessary, because it does not serve to clarify the taxonomic status of any nominal taxon.

2) “A neotype is validly designated when ... the designation is published with the following particulars: 75.3.5. evidence that the neotype is consistent with what is known of the former name-bearing type from the original description and from other sources”. There are no sources of knowledge about the holotype of *B. narilla* other than its original description, so the neotype can be compared only with the description given by Harker (1957). Besides characters common for all Baetidae, this description includes only details about the coloration of the abdomen, the proportions of hind leg segments, and the structure of genitalia. The following contradictions in characters between holotype and neotype were found:

In the holotype description, coloration is characterized as follows: “First two abdominal segments light brown, segments 3–7 yellow, the posterior segments light brown”; in the neotype description—coloration is characterized as follows: “abdominal segments 1–2 with central cream marking, 3 dark brown, 4 cream, 5–6 dark brown, 7–10 light brown”.



**Figures 1–15.** Australian *Centroptella*. **1–7** *C. illiesi*, tergallii I–VII **8–14** *C. fustipalpus* (paratype), tergallii I–VII **15** *C. fustipalpus* (holotype), right mandible.

In the holotype description, hind leg proportions are characterized as follows: “tibia and tarsus equal in length, being about three-quarters length of femur. Tarsal segments of hind leg in decreasing order of length: 2, 3, 5, 4, 1 (fused with tibia)”; in the neotype description, hind leg proportions are characterized as follows: 1.00 : 0.74 : 0.09 : 0.18 : 0.10 : 0.08 : 0.15. That means, that the neotype has a femur/tibia/tarsus

ratio of 1 : 0.75 : 0.6 (i.e., tibia and tarsus are not equal in length), and its tarsal segments in decreasing order of length are 2, 5, 3, 4, 1. The meaning of these numbers is unclear, because hind legs of all Baetidae have only 4 tarsal segments (including the first one, which is immovably fused with the tibia); but in any case, in the holotype the pen-penultimate segment is longer than the claw-bearing segment, while the neotype has the pen-penultimate segment shorter than the claw-bearing segment.

The drawing of gonostyli included with the holotype description (Harker 1957: fig. 50) does not resemble any known species, including the species described and figured in the neotype description. Words used to describe the holotype genitalia are as follows: "Forceps (fig. 50) 4-segmented; the second segment broad and short, arched on its inner surface, third segment much longer and also arched, distal segment small. Penis with a sharp spine distally (fig. 48); penis cover present". Here unistyligers were interpreted as being the first segments, so 3-segmented gonostyli ("forceps") were described as 4-segmented ones, and paraprocts were assumed to be the "penis"; thus, the only peculiar character is "penis cover present". Judging by the figure in the original holotype description, its "penis cover" is a wide outgrowth of 9<sup>th</sup> abdominal sternum, projected more distally than the unistyligers. In contrast to this, in the species to which the neotype belongs, the margin of the 9<sup>th</sup> abdominal sternum between the unistyligers is straight and non-projected (Suter and Pearson 2001: fig. 4; Webb and Suter 2010: fig. 20).

3) "A neotype is validly designated when ... the designation is published with the following particulars: 75.3.6. evidence that the neotype came as nearly as practicable from the original type locality". In the publication where this neotype was designated (Suter and Pearson 2001), a number of specimens were reported from localities much closer to the original type locality than the locality from which the neotype was collected. Thus, this neotype did not come from the nearest locality. The specimens determined as "*Bungona narilla*" and collected near the type locality, are larvae; but the paragraph 75.3.5 of the ICZN states: "a neotype may be based on a different sex or life stage, if necessary or desirable to secure stability of nomenclature".

Later, Webb and Suter (2010) restricted the concept of *B. narilla*, which they continued to regard as conspecific with *fustipalpus* [*Cloodes*], and they restored the species status of *Bungona illiesi* (Lugo-Ortiz & McCafferty, 1998).

The possibility to designate a new neotype after respective request to the International Commission of Zoological Nomenclature (according to Article 75.5 of the ICZN) can be a reasonable step for the rectification of this situation and taxonomic stability within the genus *Bungona*. Nevertheless, such a step should be taken only when new material of reared imaginal and larval specimens (preferably close to the type locality) is available. Despite considerable effort, such material is not available yet. Consequently, usage of the generic name *Bungona* is questionable and as such does not meet the requirements of the Article 23.9.1 of the ICZN.

The Australian Baetidae remain poorly known, with only 20 species described to date. Webb and Suter (2011) recognised 60 species, but most of these have not been formally described. We believe that the species originally described as *Bungona narilla* actually exists, and for this reason only this species (but not others) should bear this

generic and specific name. The fact that specimens with characteristics of *B. narilla* have not been found in the vicinity of the type locality of *B. narilla* (Coal and Candle Creek) does not mean that *B. narilla* is a wrongly described *Centroptella*, because no specimen of *Centroptella* has been found in this place either (Suter and Pearson 2001: 250). It cannot be assumed as fact that the imago and larva Harker (1957) described under the name *B. narilla* really belong to one and the same species.

Given the inadequate nature of the original description, the loss of the type material, the improper assignment of a neotype, and the poorly documented diversity of related species in Australia, *Bungona narilla* (the type species of the genus *Bungona*) should be regarded as a *nomen dubium*. It then follows that the senior generic name for the species described below should be *Centroptella*.

### ***Centroptella* Braasch & Soldán, 1980**

Figs 1–153

= *Chopralla* Waltz & McCafferty, 1987: 182, syn. nov.

= *Crassolus* Salles, Gattolliat & Sartori, 2016: 104, syn. nov.

**Type species.** *Centroptella longisetosa* Braasch & Soldán, 1980.

**Systematic position and characters.** *Centroptella* is characterized by an unusual combination of characters: on one hand, it undoubtedly belongs to the holophyletic taxon Baetovectata Kluge & Novikova, 2011, based on (1) presence of two marginal intercalaries in each space of wing (Fig. 149), (2) narrow and arched gonovectes of the penis (Figs 77–80, 141–143, 146, 150152) and (3) medially inclined subimaginal gonostyli when they are developing under the larval cuticle (Figs 79, 148). The taxon Baetovectata belongs to the holophyletic taxon Anteropatellata Kluge, 1997, which is characterized by the presence of a patella-tibial suture on the forelegs of the larva and female imago and subimago. On the other hand, the leg structure of *Centroptella* does not conform with the characteristics of Anteropatellata. The larva of *Centroptella* has the structure of the tibia modified and different on each pair of legs, so that the patella-tibial suture is absent on forelegs and greatly shifted distally on the middle and hind legs; a row of long setae, which in some other taxa forms a transverse arc, in *Centroptella* is greatly stretched along the tibia, being different on the fore, middle and hind legs (Figs 16–18, 49–51, 90–92); the female imago and subimago of *Centroptella* has the usual leg structure, with the patella-tibial suture not shifted distally, but without patella-tibial suture on forelegs. This leg structure is characteristic of the plesiomorphon Protopatellata Kluge & Novikova, 2011 and has striking similarity with the Afrotropical taxon *Potamocloeon* Gillies, 1990 (= *Maliqua* Lugo-Ortiz & McCafferty, 1997), which undoubtedly belongs to Protopatellata and has no features of Baetovectata (Kluge 2019). The Neotropical genus *Cloeodes* Traver, 1938, which some authors have confused with *Centroptella* (see above), has none of these features, and its larval and imaginal leg structure is typical for Anteropatellata (Kluge 2017).

Besides this paradoxical combination of baetovectatan and protopatellatan characters, *Centroptella* has an evident autapomorphy: secondary swimming setae on the outer sides of the larval cerci in the distal part of the cercus have oval transverse bases and form a regular row (Figs 59, 129–131); in this respect, they resemble the primary swimming setae on the inner side of the cercus (Fig. 132), but they are smaller and less densely arranged.

Another peculiar character of *Centroptella* is the presence of a pair of spaced transverse rows of long bifurcate setae on certain abdominal sterna of the larva (Figs 58, 117–119); in different species these setal rows are present on sterna II–VI or on part of them, at least on sterna IV–V. Identical setal rows are found in a few other, non-related taxa (e.g., *Potamocloeon* Gillies, 1990 and *Cloeodes* Traver, 1938).

### Status of the genus-group name *Chopralla*

Waltz and McCafferty (1987a) divided the Old World genus *Centroptella* into two parts, one of which (including the type species of *Centroptella*) they united with the New World genus *Cloeodes* Traver, 1938, and for another one established a new genus *Chopralla* with the type species *Centroptella ceylonensis* Müller-Liebenau, 1983. The genus *Chopralla* was separated from *Cloeodes* = *Centroptella* “by the absence of ventral tufts of setae on abdominal segments 2–6, the apically rounded gills (versus broadly pointed in *Cloeodes* species), the peculiar claw structure (unlike edentate claws of *Cloeodes*), and the possession of long, fine tibial seam setae (not present in *Cloeodes* species)”. Among these four characters, only the difference in claw structure exists in reality, while the other three characters were reported erroneously (Kluge 2017). At the same time, the Old World species attributed by Waltz and McCafferty to *Cloeodes*, are closely related to the species placed by them in *Chopralla*, being distant from the New World species of *Cloeodes*. Because of this, Salles et al. (2016) united these Old World species in one genus, leaving only the New World species in the genus *Cloeodes*. At the same time, they changed the generic name *Centroptella* to the name *Bungona*, which they regarded to be its senior synonym (see above), so the generic name *Chopralla* was regarded to be a junior synonym of *Bungona*. Here we recognise the generic name *Centroptella* as a valid one, thus a new formal generic synonymy is established: *Centroptella* = *Chopralla*, syn. nov. If the genus *Centroptella* is divided into subgenera, one of these subgenera should bear the subgeneric name *Chopralla* (see below).

### Status of the genus-group name *Crassolus*

The genus *Crassolus* Salles, Gattolliat & Sartori, 2016 was established for a single South African species *Crassolus inzingae* (Crass, 1947), which was originally described in the genus *Pseudocloeon* (Crass 1947) and subsequently placed in the genus *Baetis* (Gillies 1994) and then in the genus *Cloeodes* (Waltz and McCafferty 1994). The species *Pseu-*

*docloeon saxophilum* Agnew, 1961 which was originally described from the Western Cape Province, was regarded to be a junior synonym of the species *inzingae* [*Pseudocloeon*], which was originally described from Natal (Waltz and McCafferty 1994).

Examination of reared material of *saxophilum* [*Pseudocloeon*] collected in the Western Cape Province in 2019 (Figs 151, 152), reveals that this species has all the characters of *Centroptella* and is closely related to *C. ingridae* sp. nov. described below.

Salles et al. (2016) did not provide direct comparison of the new genus *Crassolus* with the genus under the name “*Bungona*”. In their phylogenetic schemes (Salles et al. 2016: figs 1, 2), the genus *Crassolus* is opposed to the whole branch comprising the genera “*Bungona*” (actually *Centroptella*) and *Cloeodes*. It seems, however, that the existence of the branch (indicated as Node 72) was not based on autapomorphies. Node 72 was characterised by five apomorphies under the numbers 3, 9, 40, 42 and 48, none of which separates it from *Crassolus*:

Character “3” (distance between prosthema and incisors of right mandible) was said to have increased from 0.00 (ancestral condition reported for *Crassolus inzingae*) to 0.04 (Node 72). Actually, according to the matrix of characters (Appendix S3), among the species attributed to “*Bungona*”, this character varies from 0.00 to 0.26. The condition “0.00” was reported for the larvae determined as “*Bungona (Chopralla) liebenauae*” and actually belongs to the new species *Centroptella ingridae* sp. nov. described below (Fig. 145).

Character “9” (length of fore femur / distance between base of fore femur and base of most distal setae of fore femur) was said to have increased from 0.92 (ancestral condition reported for *Crassolus inzingae*) to 0.95 (Node 72). Actually, according to the matrix of characters, among the species attributed to “*Bungona*”, this character varies from 0.92 (in three species included in the matrix) to 1.00.

Character “40” (slender process on prosthema of right mandible) was said to have changed from “0=absent” (ancestral condition reported for *Crassolus inzingae*) to “1=present” (Node 72). Actually, according to the matrix of characters, this process is absent in the larvae determined as “*Bungona (Chopralla) liebenauae*” and actually belongs to the new species *Centroptella ingridae* sp. nov. described below (Fig. 145).

Characters “42” and “48” are “setae between prosthema and mola of right mandible” and “long setae between prosthema and mola of left mandible”. The both characters were said to have changed from “1=present” (ancestral condition) to “0=absent” (in the Node 72). In the matrix of characters (Salles et al. 2016: Appendix S3), the condition “1=present” was reported for *Crassolus inzingae*, in contrast to all species included at Node 72 (including all species attributed to “*Bungona*”), for which the condition “0=absent” was reported. Vice versa, the diagnosis of the genus *Crassolus* (Salles et al. 2016: p. 105) included the words: “absence of long setae between prosthema and mola of both mandibles”, while the diagnosis of the genus *Bungona* (ibid., p. 100) included the words: “spine-like setae between prosthema and mola of right mandible present”. On the drawings (Salles et al. 2016: figs 4C–F) the right mandibles of *Crassolus inzingae* and *Bungona (Chopralla) ceylonensis* were shown without setae between prosthema and mola, but the right mandibles of *Bungona*

(*Bungona*) *narilla* and *Bungona* (*Centroptella*) *soldani* were shown with these setae. Actually, in all species of *Centroptella* setae between the prostheca and the mola vary individually from very small to absent (Figs 15, 40, 41, 144, 145).

The monotypic genus *Crassolus* was said to be characterized by six apomorphies under the numbers 0, 2, 5, 7, 20 and 22 (Salles et al. 2016: p. 96, fig. 1 and Appendix S2). Actually, all these characters are found among the species attributed by these authors to “*Bungona*”: Character “0” (length of body) was reported as 6.0 mm for *Crassolus* and as 2.5–6.2 mm for “*Bungona*”; according to the original description, in *Crassolus inzingae* it varies as 4.5–6.0 mm (Crass 1947). Character “2” (angle of subtriangular process of left mandible) was reported as 2.38 for *Crassolus* and as 2.35–2.70 for “*Bungona*”. Character “5” (length of fore femur / length of fore tibia and tarsus combined) was reported as 0.85 for *Crassolus* and as 0.80–1.05 for “*Bungona*”. Character “7” (length of setae on outer margin of fore femur / width of fore femur) was reported as 0.22 for *Crassolus* and 0.29–0.63 for “*Bungona*”. Character “20” (length / width of fore wing) was reported as 2.78 for *Crassolus* and as 2.39 and 2.44 for two species of “*Bungona*” with known imagines; however, in the species whose larvae were determined as “*Bungona* (*Chopralla*) *liebenauae*” (which is described here as *Centroptella ingridae* sp. nov.) this proportion is 2.94 (Fig. 149). Character 22 (number of spaces in RS sector of fore wing with marginal intercalary veins) was reported as 8 for *Crassolus* and as 8 and 0 for two species of “*Bungona*” (possibly, misprints).

The type species of *Crassolus* is closely related to *Centroptella ingridae* sp. nov. described below; these species have many common characters, including peculiar halberd-like tips of gonovectes not found in other taxa (Figs 137–143, 146, 150–152). In the original description of *Pseudocloeon inzingae*, the gonovectes were neither described, nor figured (Crass 1947: fig. 9g). In the redescription of this species under the name *Cloeodes inzingae*, gonovectes were adequately figured behind unistyligers, but with small hooks instead of the halberd-like structures (Waltz and McCafferty 1994: fig. 6). In the subsequent redescription of this species under the name *Crassolus inzingae*, the halberd-like structures were drawn, but the proximal borders of unistyligers (located externally) were drawn by interrupted lines as internal structures, probably being confused with the gonovectes (Salles et al. 2016: fig. 9D). Here the genitalia of lectotype are figured based on the photo, to show the correct position of gonovectes and outlines of unistyligers (Fig. 150).

Salles et al. (2016: appendix S3) believed that larva of *Crassolus inzingae* had no denticles on claws, in contrast to *Chopralla*. According to the original description, its “claw without denticulations” (Crass 1947: p. 62 and fig. 9e). Subsequently this character never had been checked, neither for *inzingae* [*Pseudocloeon*], nor for *saxophilum* [*Pseudocloeon*] (Agnew 1961; Waltz and McCafferty 1994). Actually, the type specimens of both species have a few small denticles by the sides of the claw, similar to that of *Centroptella ingridae* sp. nov. (Figs 126, 127) (Helen Barber James, personal communication).

Based on the above, the following generic synonymy is suggested: *Centroptella* = *Crassolus*, syn. nov. Within the genus *Centroptella*, several Asian and African species, including *C. inzingae* and *C. ingridae* sp. nov., constitute a natural species group, characterized by the halberd-like tips of the gonovectes and other common characters in imaginal and larval structure.

### Subgeneric classification of *Centroptella*

Salles et al. (2016) divided the genus *Centroptella* (under the name “*Bungona*”) into three subgenera, two Asian subgenera *Chopralla* and *Centroptella*, and one Australian subgenus under the name “*Bungona*”.

Among them, the subgenus *Chopralla* was an artificial group, because one of its species belongs to the *inzingae-ingridae* group, while another species of the *inzingae-ingridae* group was placed in a separate genus *Crassolus* (see above).

Two other subgenera, *Centroptella* and “*Bungona*” had not been separated one from another by any currently recognized characters.

According to the diagnosis of the subgenus *Bungona*, “Dorsal surface of labrum with two setae on anterolateral corner” (character “1”). In contrast, according to the original descriptions by Lugo-Ortiz and McCafferty (1998), there are 6–8 setae in *fustipalpus* [*Cloeodes*] and 3–4 setae in *illiesi* [*Cloeodes*].

According to the diagnoses of the subgenera *Bungona* and *Centroptella*, they differ by the distance between the prosthema and mola of the right mandible (character “3”). However, the structure and position of the right prosthema is the same in the larva determined as “*Bungona narilla*” and in *Centroptella soldani* (Salles et al. 2016: figs 4C, D).

According to the diagnosis of the subgenus *Centroptella*, it has “few setae on outer margin of fore femur (around six)”, in contrast to 10 in *Bungona* (character “14”). However, according to original descriptions by Lugo-Ortiz and McCafferty (1998), there are 5–8 setae in *fustipalpus* [*Cloeodes*] and 5–7 setae in *C. illiesi* [*Cloeodes*].

According to the diagnosis of the subgenus *Bungona*, it has “angle of row of long setae on posterior surface of fore tibia around 60°”, in contrast to 30° in *Centroptella* (character “13”). Actually this angle is around 30° both in the species attributed to *Bungona* (Fig. 16) and in the type species of *Centroptella* (Fig. 49), in contrast to around 60° in the species attributed to *Chopralla* (Fig. 90).

Thus, the subgeneric classification proposed by Salles et al. (2016) is inconsistent. In the present paper we accept the genus *Centroptella* (= *Chopralla* = *Crassolus*) without dividing it into subgenera.

### Composition of the genus *Centroptella*

Considering the factors discussed above, the genus *Centroptella* should be accepted as comprising the following nominal species (alphabetically): *Centroptella bifida* (Shi & Tong, 2019) comb. nov.; *Centroptella bintang* (Marle, Salles & Gattolliat, 2016) comb. nov.; *Centroptella ceylonensis* Müller-Liebenau, 1983; *Centroptella colorata* Soldán, Braasch & Muu, 1987; *Centroptella fusina* (Tong & Dudgeon, 2003) comb. nov.; *Centroptella fustipalpus* (Lugo-Ortiz & McCafferty, 1998) comb. nov.; *Centroptella illiesi* (Lugo-Ortiz & McCafferty, 1998) comb. nov.; *Centroptella inzingae* Crass, 1947 comb. nov.; *Centroptella liebenauae* Soldán, Braasch & Muu, 1987; *Centroptella longisetosa* Braasch & Soldán, 1980; *Centroptella ovata* (Shi & Tong, 2019) comb. nov.;



**Figures 16–18.** *Centroptella illiesi*, tibiae of fore, middle and hind legs, view from anterior side (bases of long setae shown both on anterior and posterior sides).

*Centroptella papilionodes* (Marle, Salles & Gattolliat, 2016) comb. nov.; *Centroptella pontica* (Sroka, Godunko & Gattolliat) (in Sroka et al. 2019) comb. nov.; *Centroptella pusilla* Müller-Liebenau, 1984; *Centroptella quadrata* (Shi & Tong, 2019) comb. nov.; *Centroptella saxophila* Agnew, 1961 comb. nov., *Centroptella similis* Müller-Liebenau, 1983; *Centroptella soldani* Müller-Liebenau, 1983. Below, a new synonymy *C. longisetosa* = *C. liebenauae* is established, and a new species, *C. ingridae* sp. nov. is described. In subsequent publications, some other synonyms will be proposed and several new species of *Centroptella* from the Oriental and Afrotropical regions will be described.

### Type specimens of *Centroptella liebenauae*

Under the name “*Centroptella liebenauae*”, Soldán et al. (1987) described two different species, one of which was described as larva, and the other as male and female imagoes. These descriptions were based on specimens collected at the same place (Suoi Bac Stream near Tam-Dao Mountain in Vietnam), but at different times: larvae described

as “*Centroptella liebenauae*” were collected in autumn 1985, and imagines described as “*Centroptella liebenauae*” were reared from larvae in spring 1982. Therefore, in order to determine the correct application of the name “*Centroptella liebenauae*”, it is necessary to examine the holotype. However, during the course of this research, we discovered some problems associated with this holotype, which we detail below. The following specimens and labels were examined by us; they are deposited in the collection of the Institute of Entomology (BC CAS) in České Budějovice, Czech Republic:

- (1) Mature male larva with labels: “VIETNAM, Vinn Phu Prov., Soui Bac – Tam Dao, 10–16.X.1985 T. Soldán”, “*Centroptella liebenauae* T. Soldán det. 1985” and “HOLOTYPE”;
- (2) 46 larvae with labels: “VIETNAM, Vinn Phu Prov., Suoi Bac Stream, Tam-Dao, 10–16.10.1984 T. Soldán”, “*Centroptella liebenauae* T. Soldán det. 1985” and “PARATYPES”; many of these larvae are late instars, and some are ready to moult to subimago;
- (3) tube with 3 male imagines (one without genitalia), 1 male subimago, 1 female imago, 1 male larval exuviae and 1 abdomen of female subimago extracted from mature larva, with labels: “VIETNAM, stream, Tam-Dao 60 km NW of Hanoi, 23–25.5.1982 T. Soldán”, “*Centroptella*, T. Soldán det. 1982” and “PARATYPE”; now larval exuviae, parts of one male imago and parts of male subimago are mounted on slides in Canadian balsam. The larval exuviae are designated as the neotype of *Centroptella liebenauae* (see below).

All specimens in tubes (1) and (2) belong to one and the same species, which is described below as *C. ingridae* sp. nov., and all specimens in tube (3) belong to a single, different species, which is *C. longisetosa*.

According to the original description, the holotype is a larva collected 23–25.V.1982 together with an additional 18 larvae and 5 reared winged insects (three male imagines, one female imago and one male subimago), while larvae collected 10–16.X.1984 and 17.X.1984 are paratypes. This means that the larva labelled as “holotype” was actually collected 10–16.X.1984 (not 16.X.1985), and is not the holotype, but a paratype. We speculate that the true holotype (i.e., the specimen designated as the holotype in the original publication) is mixed among the 18 other larvae collected on the same dates (23–25.V.1982) and now cannot be recognized among them.

Judging by the list of specimens examined in the original description, among the specimens contained in the tube (3), the male imago without genitalia is “paratype No. 1”, the single female imago is “paratype No. 2” and the single male subimago is “paratype No. 3”; the single set of male larval exuviae belongs to one of four males in this tube. The location of the 19 larvae from this series (including the true holotype) is unknown. The lost holotype was an intact larva; no structures were mounted on any slide, so its details were not examined, and the authors of the original description could not have known to which of the two species it belonged.

Sometimes larvae of different species of *Centroptella* can be collected at the same place (NJK; unpublished data). In the case of the larvae of the two species described

under the name “*Centroptella liebenauae*”, they differ in size, shape and coloration; these differences are easily visible if they lie together, but such differences can be overlooked if they are examined separately. The fact that the authors of the original description did not notice these differences indicates that they never saw larvae of these two species side-by-side. Judging by the fact that all 47 larvae collected 10–16.X.1985 belong to *C. ingridae* sp. nov., and all five reared imagines and subimagines collected 23–25.V.1982 belong to *C. longisetosa*, we assume that all larvae collected 23–25.V.1982, including the lost holotype of *C. liebenauae*, also belong to *C. longisetosa*. It is well known, that even in unimpaired rivers composition of mayfly communities varies considerably over time (e.g., Svitok 2006; Leunda et al. 2009) and has well-expressed seasonality in Oriental streams (e.g., Dudgeon 1984); it follows that the conditions in the Suoi Bac Stream are likely to be different in May and in October, and between the years 1982 and 1985.

### Neotype designation for *Centroptella liebenauae*

Complete set of last instar male larval exuviae (Figs 26–32, 47, 52–59, 76) with the geographical label “VIETNAM, stream, Tam-Dao 60 km NW of Hanoi, 23–25.5.1982 T. Soldán” is designated here as the **neotype** of *Centroptella liebenauae*. All part of these exuviae are mounted on slide in Canada Balsam, except for abdomen and tergali, which are mounted on the same object glass in dry condition, under a separate cover glass. These exuviae belong to one of the four males—three imagines (Fig. 80) and one subimago, but is unclear to which because the rearing was not individual. Each of these three male imagines and one male subimago are labelled now as “possibly from neotype”. The neotype (male larval exuviae) and all four-winged male specimens, each of which can belong to the neotype, as well as female imago in the same tube, will be permanently deposited in the Institute of Entomology (BC CAS) in České Budějovice, Czech Republic.

### New synonymy caused by neotype designation

Based on this neotype designation, we propose a new synonym: *Centroptella longisetosa* = *Centroptella liebenauae* syn. nov.; another species, described under the name “*Centroptella liebenauae*”, is a new species, and it is described here under the name *C. ingridae* sp. nov. (see below).

### Reasons for the neotype designation

According to the Article 75.3 of the International Code of Zoological Nomenclature, a neotype is validly designated when there is an exceptional need and only when that need is stated expressly and when the designation is published with the particulars listed in the paragraphs 75.3.1–75.3.7. In the present case, such exceptional need is

present, because usage of the same name *Centroptella liebenauae* for two distant species causes confusion; all particulars required in the paragraphs 75.3.1–75.3.7 are published here as the following:

- “75.3.1. a statement that it is designated with the express purpose of clarifying the taxonomic status ...”. This purpose is to choose, which of two different species originally described under the name *Centroptella liebenauae*, should bear this name.
- “75.3.2. a statement of the characters that the author regards as differentiating from other taxa the nominal species-group taxon for which the neotype is designated ...”  
These characters are give below, in the discription of *C. longisetosa*.
- “75.3.3. data and description sufficient to ensure recognition of the specimen designated”. These data are given above.
- “75.3.4. the authors’ reasons for believing the name-bearing type specimen(s) (i.e., holotype, or lectotype, or all syntypes, or prior neotype) to be lost or destroyed, and the steps that had been taken to trace it or them”. The steps that had been taken to trace the holotype, are reported above; the lost holotype is an intact larva, whose individual features have never been reported or figured; even its sex is unknown. If this specimen is found in future, it will be impossible to prove that it is the holotype designated in the original publication, as it could be any other specimen among 19 lost larval specimens, which have one and the same geographical label.
- “75.3.5. evidence that the neotype is consistent with what is known of the former name-bearing type from the original description and from other sources ...”. The original description contains characters and figures of two different species, *C. longisetosa* and *C. ingridae* sp. nov. Based on the original description, we know that the former name-bearing type was collected in spring 1982, and analyzing the collection we know that all specimens collected at that time belong to *C. longisetosa*. Designating the neotype from specimens collected in autumn 1985 and belonging to a species different from the holotype, would clearly violate this paragraph.
- “75.3.6. evidence that the neotype came as nearly as practicable from the original type locality ...”. The neotype comes from the type locality and has the same date of collecting as the holotype.
- “75.3.7. a statement that the neotype is, or immediately upon publication has become, the property of a recognized scientific or educational institution, cited by name, that maintains a research collection, with proper facilities for preserving name-bearing types, and that makes them accessible for study”. This institution is the Institute of Entomology (BC CAS) in České Budějovice, Czech Republic, where this specimen was deposited recently.

Besides these formal rules, the Code requires maintainance of prevailing usage of the taxa names which can be done only under plenary power of the Commission (paragraph 75.6). In this case, referring to prevailing usage is impossible, because there are only six publications where the species name *liebenauae* [*Centroptella*] has been mentioned (Soldán et al. 1987; Soldán 1991; Tong and Dudgeon 2003; Salles et al.

2016; Sroka et al. 2019; Shi and Tong 2019). Among them, reports of larvae under this name given by Soldán (1991) and by Shi and Tong (2019) do not contain original taxonomic conclusions. Soldán et al. (1987) and Salles et al. (2016) applied this name both to *C. longisetosa* and *C. ingridae* sp. nov. at the same time; the phylogenetic reconstruction proposed by Salles et al. (2016) was based on a matrix of characters which contained larval characters of *C. ingridae* sp. nov. and imaginal characters of *C. longisetosa* under the common name “*Bungona (Chopralla) liebenauae*”. As a result, the classification based on this phylogenetic reconstruction was unnatural, and the closely related species *C. inzingae* and *C. ingridae* sp. nov. would be placed in different genera (see above, Status of the genus-group name *Crassolus*). Tong and Dudgeon 2003 used imaginal characters of what they called “*Chopralla liebenauae*” to confirm species identity of the newly described species *Chopralla fusina*; in this case the name “*Chopralla liebenauae*” was applied to *Centroptella longisetosa*. Sroka et al. (2019), vice versa, used larval characters of what they called “*Bungona (Chopralla) liebenauae*” to confirm species identity of the newly described species *Bungona (Chopralla) pontica*; in this case the name “*Chopralla liebenauae*” was applied to *Centroptella ingridae* sp. nov. Thus, the name *liebenauae* [*Centroptella*] has been equally often applied to both *C. longisetosa* and *C. ingridae* sp. nov.

Our choice of the neotype, being the single one is consistent with the Code. Moreover, the single one provides the both considered species with monosemantic valid names, because the non-monosemantic (equivocal) name *C. liebenauae* becomes invalid. The designation of a neotype and the new synonymy proposed here will stop further confusion caused by inaccurate descriptions of two different species under the one name, *C. liebenauae*.

### **Type specimens of *Centroptella longisetosa***

The type species of the genus *Centroptella*, *C. longisetosa*, was originally described based on larvae from China. According to the original description (Braasch and Soldán 1980), the holotype of *C. longisetosa* is “Larve (Präparat in Kanadabalsam mit Tellosolve). VR China, Liu Chui, Fluß im Kuj Fon Shan; 11.XII.1959, leg. I. Hrdý”. Besides the holotype, six larvae were reported from the same locality. The place of deposition of the type material was reported as “Holotypus und 5 Paratypen in der Coll. Soldán, Praha; 1 Paratypus in der Coll. Braasch, Potsdam”.

One of us (RJG) examined the mayfly collection deposited in the Institute of Entomology (BC CAS) and could not find the slide with the holotype of *C. longisetosa*. Consultations with Tomáš Soldán also did not clarify the fate of the holotype. Instead, there is a tube with larva in alcohol with labels: “CHINA, Liu Chiu, Kuj Fon Shan Mt., stream, 11.12.1959, leg. Ivan Hrdý”, “*Centroptella longisetosa* T. Soldán det. 1980” and “HOLOTYPE”. Judging from the original description, this intact larva is not the holotype, but one of five paratypes deposited in the Soldán collection. Another paratype was reported by Waltz and McCafferty (1987a, 1987b) and by Tong et al. (2003), as a male

larva in alcohol, “deposited Purdue Entomological Research Collection, originally from paratypes in the collection of T. Soldán”. This specimen is present in the collection and has been assigned number “PERC-0063355” (Luke M. Jacobus, personal communication). Waltz and McCafferty (1987a) reported the collection data as: “Peoples Republic of China, Liu Chui, Kuj Fon Shan River, 11-12-1959, I. Hrdy.” The collection label, however, says: “China: Liu Chui river at Kuj Fon Shan 11.12.59 leg. I Hrdý”.

Waltz and McCafferty (1987b) reported that the specimen in the Purdue University Entomological Research Collection “had absorbed the ink used in labelling and is entirely black and devoid of pattern”. The specimen deposited in the Entomological Institute is also black, but this cannot be a result of ink absorption; possibly, it is a preservation artefact from vinegar acid having been added to alcohol at some point.

### ***Centroptella longisetosa* Braasch & Soldán, 1980**

Figures 19–32, 40–82

*Centroptella longisetosa* Braasch & Soldán, 1980: 123 (larva)

*Cloeodes longisetosus*: Waltz and McCafferty 1987a: 179 (larva); 1987b: 201, 206 (larva); Tong et al. 2003: 669 (larva, ♂ & ♀ imago)

*Bungona* (*Centroptella*) *longisetosa*: Salles et al. 2016: 104, figs 6B, C, K, 7F, 9C (larva, ♂ imago); Shi and Tong 2019: 572, figs 1–5 (larva)

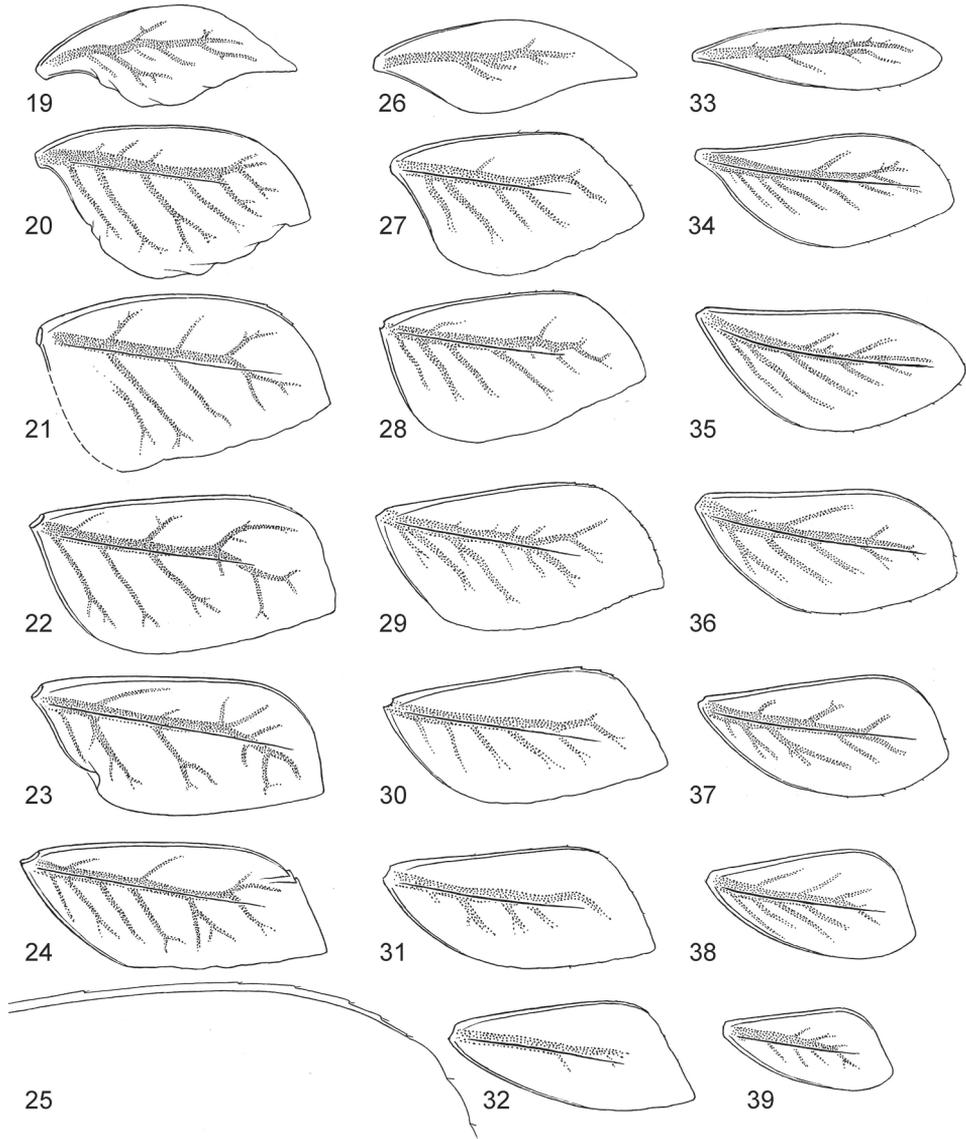
*Centroptella liebenauae* Soldán, Braasch & Muu, 1987: 342 (partim: ♂ & ♀ imagines, ♂ subimago; non larva), syn. nov.

*Chopralla liebenauae*: Tong and Dudgeon 2003: 19 (comparison of ♂ imago)

*Bungona* (*Centroptella*) *liebenauae*: Salles et al. 2016: Appendix S3 (partim: imaginal characters 20–30 and 122–131)

**Material examined. Paratypes of *Centroptella longisetosa*** (deposited in the Institute of Entomology, BC CAS, České Budějovice and Purdue University Entomological Research Collection, West Lafayette, Indiana, USA): mature female larva with labels: “CHINA, Liu Chiu, Kuj Fon Shan Mt., stream, 11.12.1959, leg. Ivan Hrdý”, “*Centroptella longisetosa* T. Soldán det. 1980”, “HOLOTYPE” (now parts of this specimen are mounted on 2 slides, eggs mounted for SEM; one middle larval leg of another specimen, in the same tube (now treated by alkali and mounted on separate slide); one larva, Peoples Republic of China, Liu Chui, Kuj Fon Shan River, 11-12-1959, I. Hrdy, PERC-0063355.

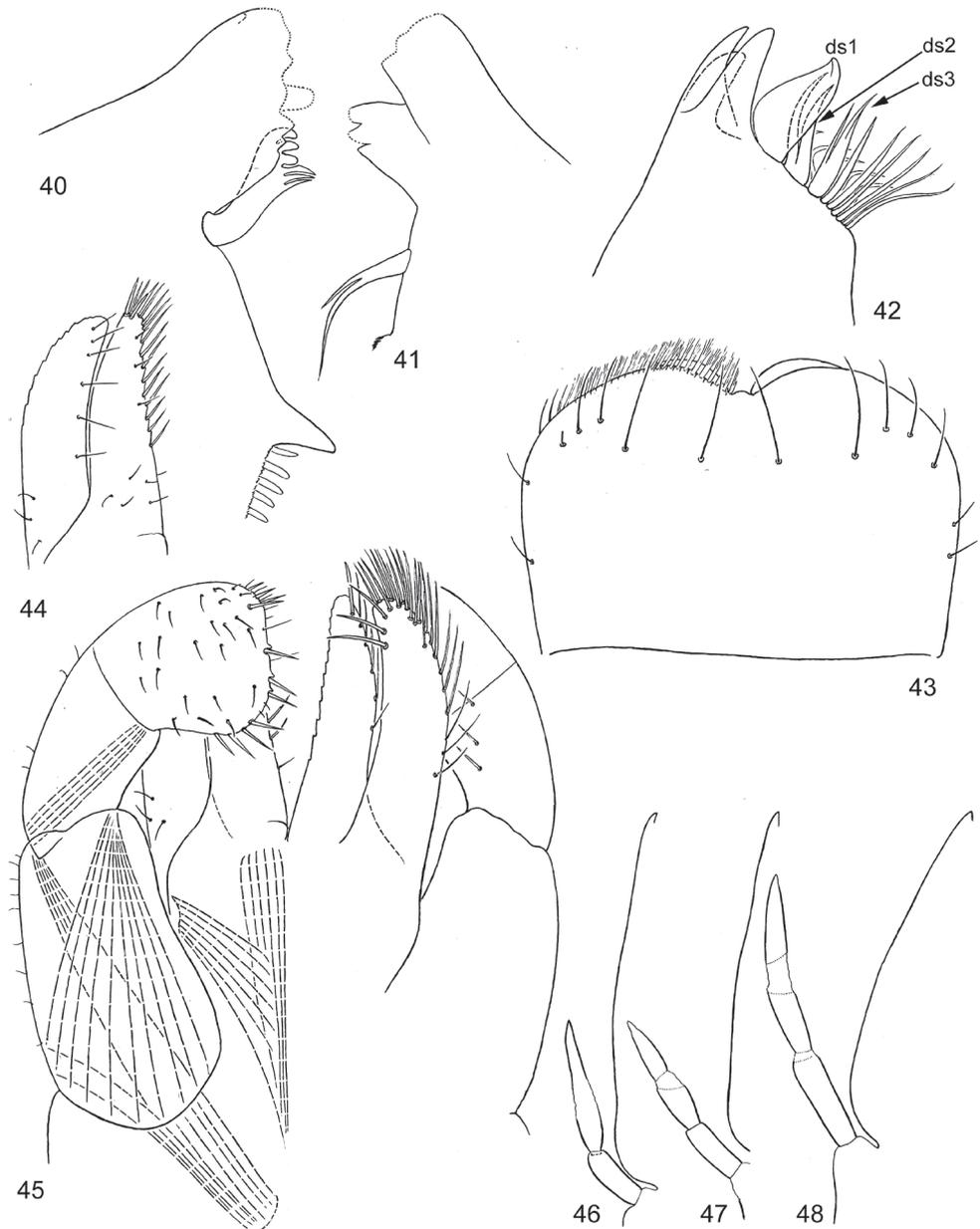
**Neotype and paratypes of *Centroptella liebenauae*** (deposited in the collection of the Institute of Entomology, BC CAS, České Budějovice): one tube containing: 3 male imagines (one without genitalia), 1 male subimago, 1 female imago, 1 male larval exuviae (neotype) and 1 abdomen of female subimago extracted from mature larva, with labels: “VIETNAM, stream, Tam-Dao 60 km NW of Hanoi, 23–25.5.1982 T. Soldán”, “*Centroptella*, T. Soldán det. 1982” and “PARATYPE”; now larval exuviae (neotype) and parts of male imago and male subimago (one of which possibly was reared from the neotype) are mounted on slides.



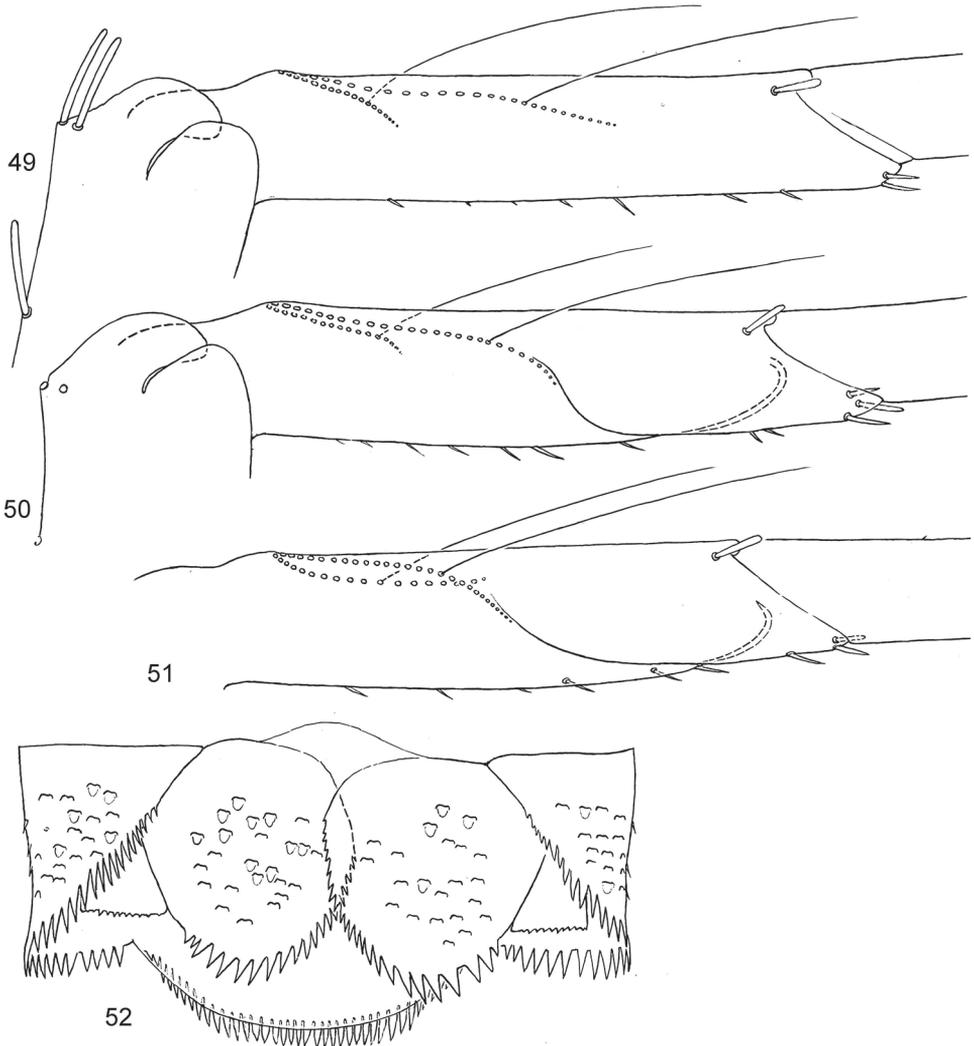
**Figures 19–39.** Asian *Centroptella*. **19–32** *C. longisetosa*: (**19–24**) tergalii I–VI of paratype of *C. longisetosa* (**25**) the same, apex costal rib of tergalium IV (**26–32**) tergalii I–VII of neotype of *C. liebenauae* (actual *C. longisetosa*) **33–39** *C. ingridae*, tergalii I–VII of sp. nov.

**Additional material.** INDIA, Tamilnadu, Tirunelveli District, Courtallam, Chittar River near Peraruvi (= Main Falls), 3–7.II.2013, coll. N. Kluge & L. Sheyko: 3 L-S-I♂, 1 L-S-I♀, 1 S-I♀, 1 L/S♂, 1 L/S♀, 1 larva of penultimate instar.

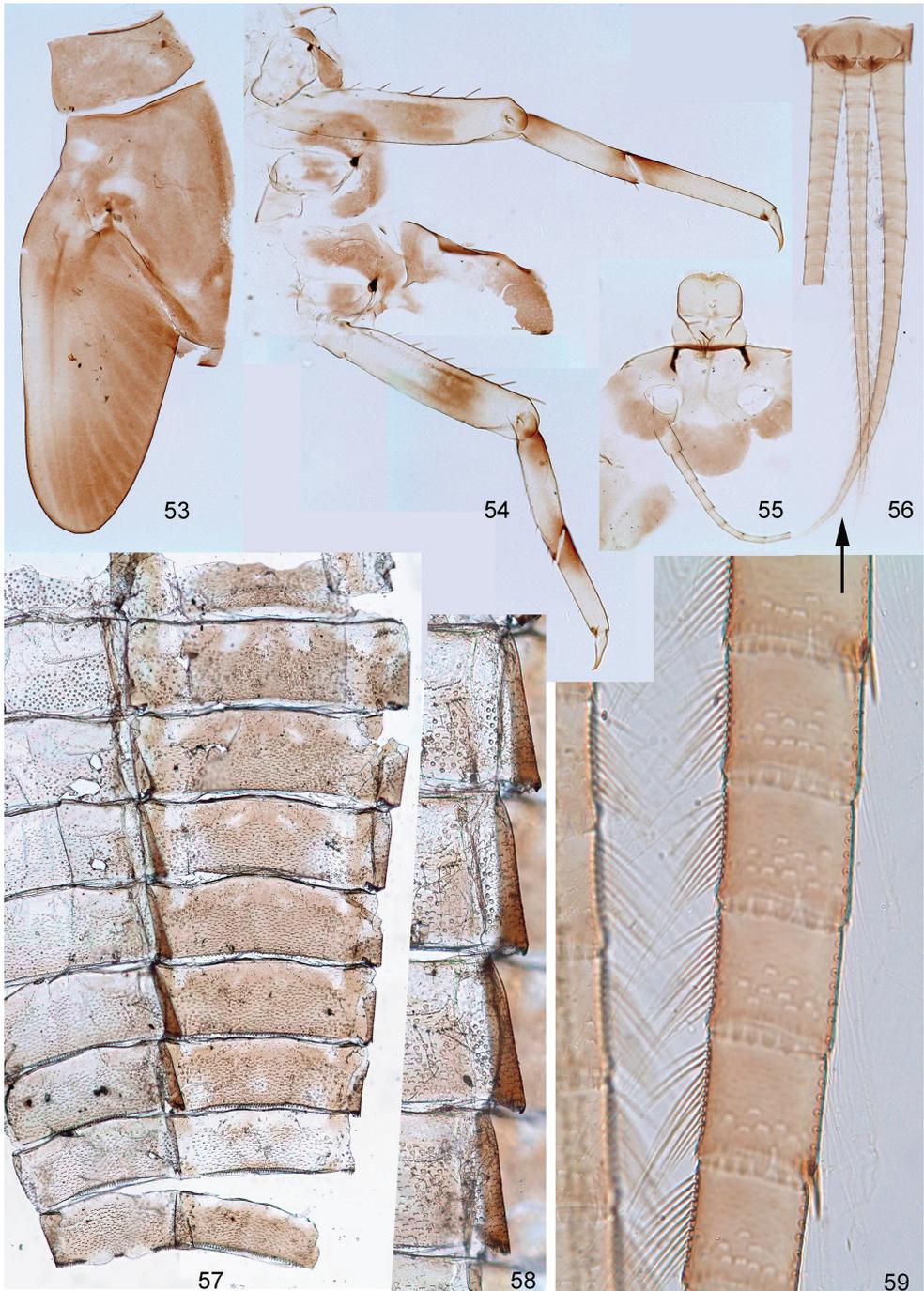
**Descriptions. Larva. Cuticular coloration.** Head mostly brown (Fig. 55); specimens from India mostly colourless, but with frons brown. Pronotum and mesonotum brown with diffuse lighter and darker areas (Fig. 53). Thoracic pleura and metanotum



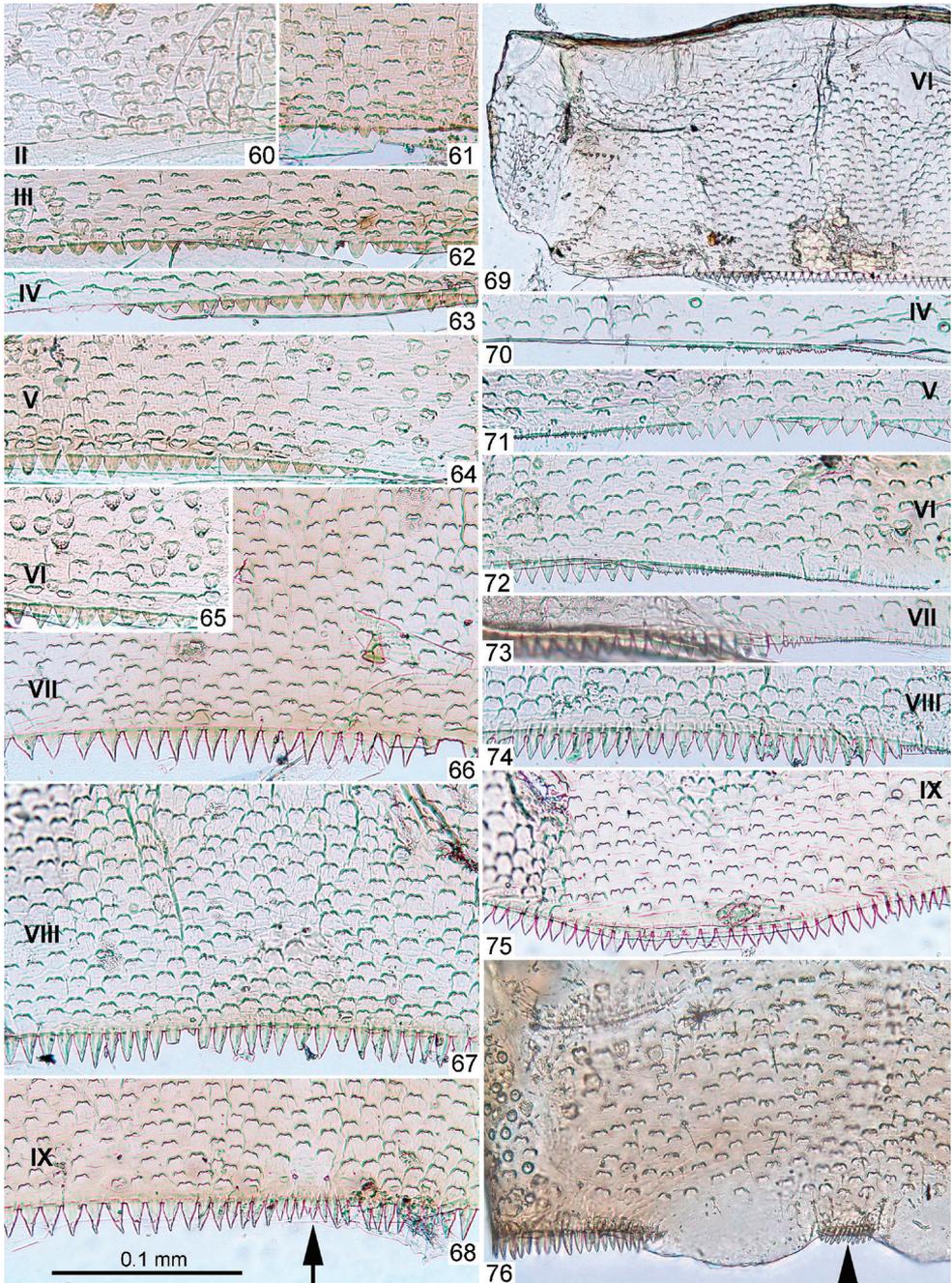
**Figures 40–48.** *Centropsetta longisetosa*. **40–46** paratype of *C. longisetosa*: **(40, 41)** left and right mandibles **(42)** apex of maxilla (**ds1, ds2, ds3** dentisetae) **(43)** labrum **(44)** glossa and paraglossa (ventral view) **(45)** labium (at left ventral view, at right dorsal view; muscles shown by interrupted lines) **(46)** maxillary palp **(47)** the same, neotype of *C. liebenauae* (actually *C. longisetosa*) **(48)** the same, specimen from India.



**Figures 49–52.** *Centroptella longisetosa*. **49–51** paratype of *C. longisetosa*: tibiae of fore, middle and hind legs, view from anterior side (bases of long setae shown both on anterior and posterior sides) **52** neotype of *C. liebenauae* (actually *C. longisetosa*): tenth abdominal segment without caudalii, ventral view.



**Figures 53–59.** Larval exuviae of *Centropsettella longisetosa* (neotype of *C. liebenauae*). **53–57** at the same magnification: **(53)** left half of pro- and mesonotum **(54)** thoracic pleura, left half of metanotum, fore- and hind legs **(55)** frons, antenna and labrum **(56)** tenth uromere and caudalii; **(57)** abdominal sterna and terga I–IX **58** margins of abdominal sterna IV–VII **59** cercus.



**Figures 60–76.** Larval exuviae of *Centroptella longisetosa*. **60–75** female, paratype of *C. longisetosa*: (**60–68**) fragments of abdominal terga II–IX (indicated by Roman numbers) (**69**) sternum VI (**70–75**) fragments of abdominal sterna IV–IX (indicated by Roman numbers) **76** male, neotype of *C. liebenauae*: fragment of abdominal sternum IX. Arrows on Figs 68 and 76 show median line.

partly brown, partly colourless; sterna colourless (Fig. 57). Forecoxa colourless; middle and hind coxa laterally brown, medially colourless; femur of each leg light, with large, diffuse, brown macula on posterior surface; tibia of each leg light at middle, at base and apex diffusely tinged with brown; tarsus of each leg proximally brown, with gradation to colourless distally; claws colourless (Fig. 54). Abdominal terga brown with lighter areas; some terga with light medioanterior sigilla; terga IV and VIII lighter than others (Fig. 57). Caudalii colourless (Fig. 56).

*Shape and setation.* Frontal suture short, nearly semicircular (Fig. 55). Labrum equally wide at base and middle, with pair of submedian long setae, 3–4 pairs of sublateral long setae and pair of long setae between submedian and sublateral ones (Fig. 43). Prosthema of left mandible with 3 blunt processes and 3 pointed processes (Fig. 40). Prosthema of right mandible directed medially or medially-proximally, bifurcate, with branches diverging under acute angle and longest branch directed proximally (Fig. 41). Maxillary canines and distal dentiseta stout; distal dentiseta widened, with apex somewhat hooked toward canines (Fig. 42). Maxillary palp in specimens from China and Vietnam short, either 2-segmented, or indistinctly 3-segmented (Figs 46, 47); in specimens from India long and distinctly 3-segmented (Fig. 48). Labium with glossae and paraglossae subequal, both narrowed apically (Figs 44–45). Glossa ventrally with irregularly arranged setae in proximal part and 4–6 setae forming ventro-median row. Paraglossa with latero-apical setae forming one regular row and few (2–4) setae just dorsal of it; with 4–6 setae in ventro-median row; with 3 setae in dorso-median row. Distal segment of labial palp widened apically (Fig. 45).

All thoracic terga without protuberances. Metanotum with vestiges of hind pro-  
toptera (Fig. 54; Tong et al. 2003: fig. 7). Femora of all legs equal, tibia and tarsus on foreleg longest, on hind leg shortest; on foreleg tarsus longer than tibia, on middle and hind legs tarsus shorter than tibia (Fig. 54); in paratype length of femur / tibia / tarsus / claw of foreleg (mm) 0.75 : 0.48 : 0.54 : 0.13; on middle leg 0.75 : 0.47 : 0.44 : 0.13; on hind leg 0.75 : 0.44 : 0.41 : 0.13. Femur parallel-sided; outer margin straight or slightly concave, apically with blunt-angled projection bearing two subapical setae; inner margin slightly convex (Fig. 54). Outer side of femur with row of 5–7 long blunt setae and 2 subapical setae of same form (Fig. 49). Inner-dorsal side of forefemur with few stout setae, these setae being half length of setae on dorsal side; middle and hind femora with minute setae only. Foreleg without patella-tibial suture, middle and hind legs with patella-tibial suture greatly shifted to apex of tibia. Posterior arm of U-shaped row of long setae on fore- and middle tibiae oblique and directed more longitudinally than transversely (Figs 49–50); on hind leg longitudinally (Fig. 51). Inner margin of tibia and tarsus with irregular, small, stout, pointed setae. Outer-apical seta of tibia blunt and elongate (Figs 49–51). Dorsal side of each tarsus with long, fine setae, situated irregularly and partly forming two longitudinal rows. Claw without denticles.

Scales on abdominal terga and sterna numerous, short, semicircular, colourless and delicate (Figs 52, 60–76). Posterior margin of abdominal tergum I smooth, without denticles; posterior margins of terga II–VI partly without denticles, partly with short semicircular and triangular denticles; terga VII–IX with longer, triangular denticles

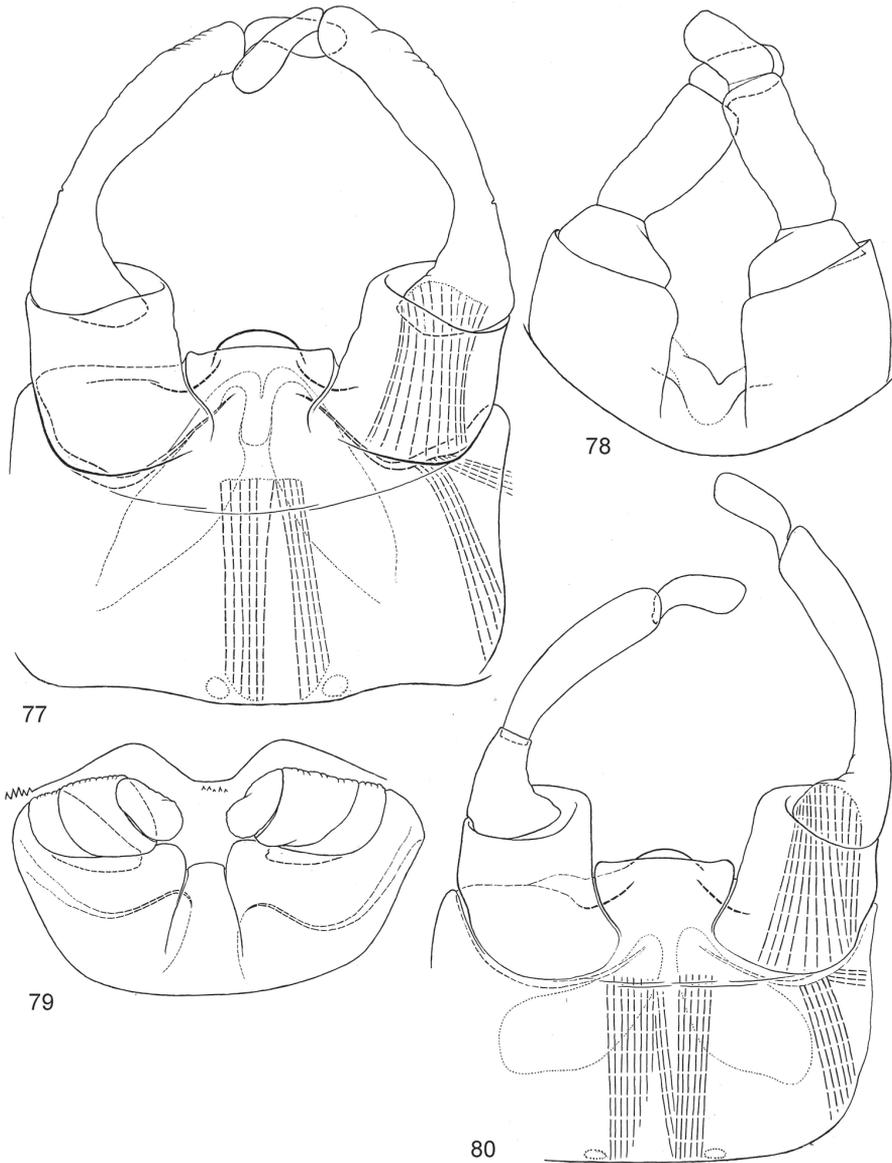
(Figs 60–68); on tergum IX denticles located behind pair of submedian setae, smaller and denser than others (but row of denticles not interrupted at this place) (Fig. 68). Posterior margin of tergum X with even row of small, narrow, pointed denticles (Fig. 52). Posterior margins of abdominal sterna I–III smooth; posterior margin of sternum IV with few, minute denticles (Fig. 70); posterior margins of sterna V–VIII with regular pointed denticles, increasing in length from sternum V to sternum VIII (Figs 71–74). Posterior margin of sternum IX in female convex, with even row of triangular denticles (Fig. 75), in male with narrow and dense denticles between protogonostyli and by sides of them (Fig. 76). Each sternum IV–VI with pair of regular, transverse rows of long, fine, bifurcate setae with spaced sockets; other sterna either without such setae, or with few, smaller setae irregularly situated (Figs 57–58). Paraprocts without anterior median apodeme, with many small, pointed denticles on posterior margin, with scales as on sterna and terga (Fig. 52).

Tergalii apically pointed and sharply differentiated as follows: tergalium I lanceolate, slightly bent, widened at midlength, with apex stretched and narrowly pointed (Figs 19, 26); tergalium II especially wide, widest at proximal half, with anal margin more convex than costal margin (Figs 20, 27); tergalia III–VI with gradation of shapes (Figs 21–24, 28–31); tergalium VII narrow, widest at distal half, with costal margin more convex than anal margin (Fig. 32). Each tergalium II–VII, besides costal and anal ribs, with straight and narrow middle rib, located on dorsal surface on background of main trachea. Costal margin with poorly expressed serration (Fig. 25); anal margin without serration; outer margin free of ribs, slightly notched, with small seta in each notch. Lateral side of each cercus with several long, pointed denticles on each 4<sup>th</sup> segment (Figs 56, 59). Each cercus, besides regular row of primary swimming setae on inner side, with smaller and thinner secondary swimming setae on outer margin; on most part of cercus secondary swimming setae with wide transverse oval bases, forming regular row (Fig. 59); on proximal 1/5 of cercus secondary swimming setae with small round bases and situated irregularly.

*Male genitalia* (examined in Indian specimen): In last larval instar, developing subimaginal gonostyli folded under larval cuticle in peculiar pose, with 3<sup>rd</sup> segments bent medially-proximally (Fig. 79).

*Subimago*. Adequately described by Soldán et al. (1987). Additional details: On all legs of male and female, all tarsal segments entirely covered with pointed microlepidetes (as in Fig. 137).

*Imago, male*. Adequately described by Soldán et al. (1987). Additional details: Length of femur, tibia and tarsal segments (mm) on foreleg 0.8 : 1 : 0.04 : 0.55 : 0.4 : 0.21 : 0.12, on hind leg 0.55 : 0.54 : 0.15 : 0.09 : 0.04 : 0.1. Tarsus of middle and hind leg with one apical spine on initial 3<sup>rd</sup> tarsomere (next after 1<sup>st</sup>+2<sup>nd</sup> tarsomere) (as in Fig. 137). Genitalia: Figs 77, 80. Sterno-styliger muscle present. Area between unistyligers forms well-outlined, trapezoid, colourless plate with distal margin widest; distal margin shallowly convex at middle and shallowly concave laterally, forming well-expressed angles adjacent to unistyligers. Gonostylus with 1<sup>st</sup> segment short and conic; 2<sup>nd</sup> segment thickened toward apex; 3<sup>rd</sup> segment elongate, narrow and thickened to-



**Figures 77–80.** Male genitalia of *Centropsettella longisetosa*. **77–79** specimens from India: **(77)** genitalia of imago **(78)** subimaginal exuviae **(79)** subimaginal gonostyli crumpled under larval cuticle **(80)** paratype of *C. liebenauae* (possibly reared from neotype): genitalia of imago.

ward apex, with proportions varying individually (Fig. 77). Penial bridge medially with semicircular, sclerotized, colourless projection and with pair of small, oblique, arched, sclerotized ridges proximad of it. Gonovectes shallowly bent, narrowed toward apices.

**Imago, female.** Adequately described by Soldán et al. (1987). Additional details: Patella-tibial suture present on middle and hind legs, absent on forelegs (as in male).

Tarsus of each leg with one apical spine on initial 3<sup>rd</sup> tarsomere (on foreleg—on tarsomere next after 2<sup>nd</sup> tarsomere, on middle and hind leg—on tarsomere next after 1<sup>st</sup>+2<sup>nd</sup> tarsomere) (as in Fig. 136).

**Eggs** (extracted from mature female larva, paratype of *C. longisetosa*). Oval, chorion with numerous irregular small protuberances (Figs 81, 82).

**Dimension.** According to original descriptions, specimens from Vietnam (type series of *C. liebenauae*) smaller, 3.7–4.3 mm; specimen from China (type series of *C. longisetosa*) larger, 3.9–5.2 mm.

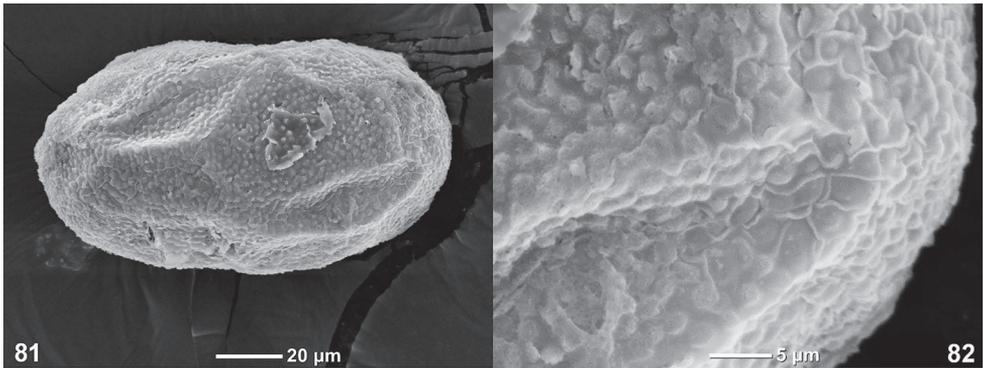
**Variability.** All 8 examined specimens from India have maxillary palp relatively long (about 0.8 of lacinia length), while specimens from China and Vietnam have maxillary palp shorter (0.5–0.6 of lacinia length, Figs 46–48). In all other respects larvae from India have the same structure as larvae from China and Vietnam, and we were unable to find any differences between them, other than the size of maxillary palp. Imagines reared from larvae of the Indian form, are indistinguishable from imagines of the typical form, and have the same unusual stylicher structure. Possibly, the examined specimens from India belong to a separate geographical form, which can be considered as a separate subspecies of the species *C. longisetosa*.

**Remarks about descriptions and figures.** The original description of *C. longisetosa* contains some errors. Instead of foreleg (Braasch and Soldán 1980: fig. 2, “Vorderbein”), middle leg is shown, as evidenced by the presence of the patella-tibial suture (Fig. 50); the text also refers to the middle tibia (“Tibia ... wenig länger als der Tarsus”), while foretibia is shorter than tarsus (Fig. 54). Tergalium of first pair is wrongly figured (Braasch and Soldán 1980: fig. 5); probably, this drawing was made from tergalium of sixth pair (Fig. 30). Labrum (Braasch and Soldán 1980: fig. 8) has wrong shape and demonstrates posterior surface. Maxilla is wrongly drawn and characterized as “Maxille (Fig. 11) apikal dreizählig”; actually, it has four apical denticles, if one regards the first dentisetula to be one of these denticles (Fig. 42).

Waltz and McCafferty (1987b) examined the paratype of *C. longisetosa* (see above) and wrote that “The secondary row of tibial setae as illustrated for *C. soldani* (figs 3i and j of Müller-Liebenau 1983) is not present in *C. longisetosus* contrary to the data indicated in table 2 of Müller-Liebenau 1983”. Probably, under the “secondary row” they mean that arm of the U-shaped tibial row, which is located on the posterior side of the tibia; this posterior arm is present in *C. longisetosa* (Figs 49–51), as well as in all other *Centroptella*.

Tong et al. (2003) redescribed the larva of *C. longisetosa* and described its imagines. Their figures, including figures of tergalia III, V and VII agree well with the species described here, but tergalium I is figured incorrectly with a rounded apex (Tong et al. 2003: fig. 8).

The larval metanotum is figured by Tong et al. (2003: fig. 7) with a vestige of hind proteron; based on larvae from the same series, Salles et al. (2016: fig. 6K) figured it without these vestiges. In all eight larvae of the last instar examined by us, including a paratype of *C. longisetosa* and the neotype of *C. liebenauae*, vestiges of hind proteron are present (Fig. 54).



**Figures 81–82.** Egg extracted from larva – paratype of *Centroptella longisetosa*.

On the figure of male imaginal genitalia (Tong et al. 2003: fig. 14), the trapezoid plate between unistyligers is correctly figured ventrad of the semicircular penial projection, but on the figure by Salles et al. (2016: fig. 9C), made from a specimen of the same series, this trapezoid plate is wrongly figured dorsad of the semicircular projection.

***Centroptella ingridae* sp. nov.**

<http://zoobank.org/0D1B2BEB-824D-4881-B219-29635BBB5C1D>

Figures 33–39, 83–149

*Chopralla* sp.: Waltz and McCafferty 1987a: 183 (larva in list of material examined)

*Centroptella liebenauae*: Soldán et al. 1987: 342 (partim: larva, non imago)

*Bungona* (*Chopralla*) *liebenauae*: Salles et al. 2016: 104, fig. 6D (larva), Appendix S3 (partim: larval characters 0–19 and 31–121); Sroka et al. 2019: fig. 6B (larva); Shi and Tong 2019: 582, figs 60–67 (larva)

**Material examined. Holotype:** L-S-I♂ {specimen [XV](1)2015}, THAILAND, Mae-Hong-Son Province, Pai, Mhor-Phaeng Falls, 11.II.2015, coll. N. Kluge & L. Sheyko (ZIN). **Paratypes:** the same locality and collectors, 9–11.II.2015: 1 L-S♂, 2 L-S-I♀, 2 L/S♂, 6 larvae (ZIN); Pai, 19–25.XI.2010, coll. K. Tomkovich: 1 I♂ (ZIN). VI-ETNAM, Vinn Phu Prov., Suoi Bac Stream, Tam-Dao, 10–16.10.1984 T. Soldán: 47 larvae (paratypes of *Centroptella liebenauae*, including the specimen wrongly labeled as “holotype”, see above) (deposited in Institute of Entomology, BC CAS, České Budějovice).

**Etymology.** This species is named in honour of Ingrid Müller-Liebenau.

**Descriptions. Larva. Cuticular coloration.** Frontal side of head colourless (Fig. 86). Pronotum and mesonotum with contrasting brown, ochre and/or colourless areas, forming characteristic pattern (Figs 83–85). Thoracic pleura and metanotum partly brown, partly colourless; sterna colourless (Fig. 89). Each leg with coxa and trochanter colourless; femur either entirely colourless, or with diffuse brown macula in distal



**Figures 83–89.** *Centroptella ingridae* sp. nov., larvae. **83–85** specimens from Thailand **86–89** paratypes of *C. liebenauae* (actually *C. ingridae* sp. nov.). Arrows show pair of protuberances on pronotum.

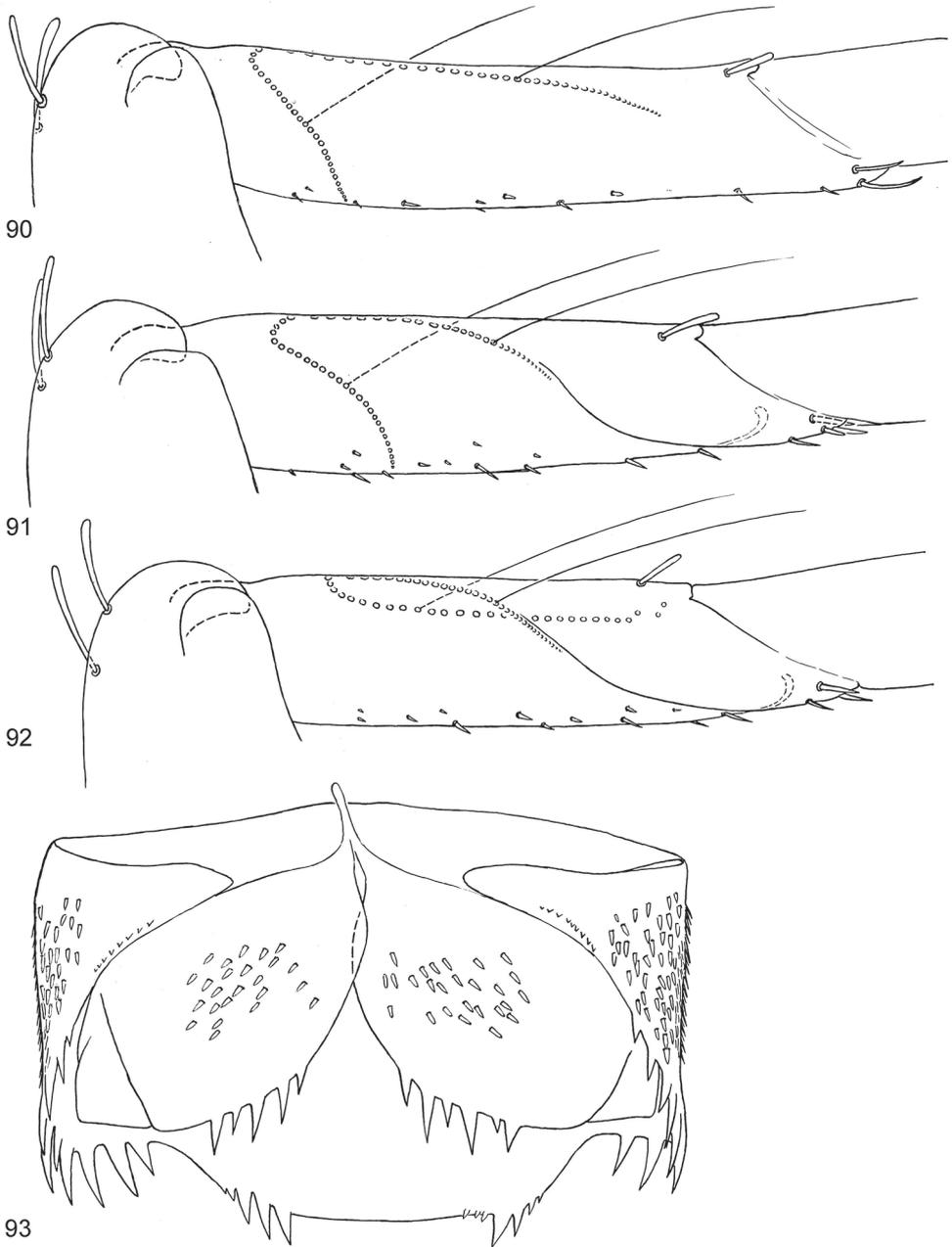
part on posterior and/or anterior surfaces; tibia and tarsus with more or less expressed diffuse brown coloration, mainly on outer side; claws brownish (Fig. 89). Abdominal terga with contrasting brown, ochre and/or colourless, areas forming characteristic pattern; most terga with large, paired, transverse blanks, which occupy medioposterior

sigilla and stretch laterally from them (Figs 83–85). Caudalii colourless at base, diffusely darkened at middle (Fig. 85).

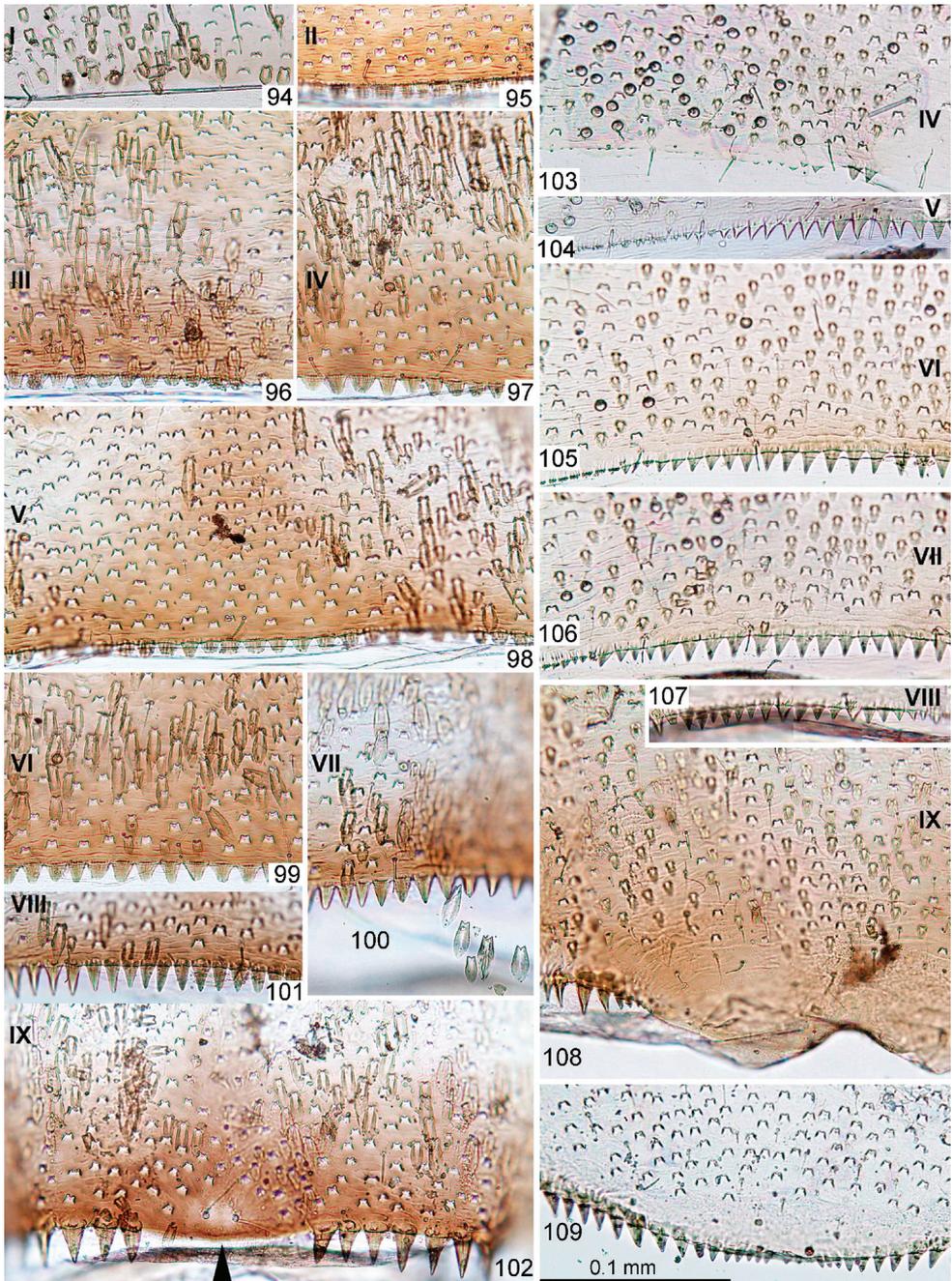
*Shape and setation.* Frontal suture short, nearly semicircular (as in Fig. 55). Labrum equally wide at base and middle, with pair of submedian, long setae, 2–3 pairs of sublateral, long setae and pair of long setae between submedian and sublateral ones (Fig. 116). Prosthema of left mandible with 3 blunt processes and 2–4 pointed processes (Fig. 144). Prosthema of right mandible directed medially-distally, with short, apical denticles and without long branch; median margin of right mandible proximad of prosthema either without processes, or with small seta-like processes (Fig. 145). Maxillary canines and distal dentiseta stout; distal dentiseta widened, with apex somewhat hooked toward canines (as in Fig. 42). Maxillary palp nearly as long as lacinia, 2-segmented. Labium with glossae and paraglossae subequal, both narrowed apically (Fig. 124). Glossa ventrally with irregularly arranged setae in proximal part and about 10 setae forming ventro-median row. Paraglossa with latero-apical setae forming one regular row and few setae just dorsad of it; with about 8 setae in ventro-median row; with 4 setae in dorso-median row. Distal segment of labial palp rounded apically (Fig. 123).

Pronotum with pair of protuberances near posterior margin (Figs 87, 88, 111; the same character listed by Shi and Tong 2019: 583, figs 62, 63 under species name *C. liebenauae*). Metanotum with vestiges of hind protoptera (as in Fig. 54). Forelegs longest, hind legs shortest; on all legs, tarsus (measured on outer side) longer than tibia; in holotype length of femur / tibia / tarsus / claw of foreleg (mm) 0.88 : 0.48 : 0.57 : 0.16; on middle leg 0.83 : 0.42 : 0.48 : 0.16; on hind leg 0.80 : 0.39 : 0.43 : 0.16. Femur parallel-sided; outer margin straight or slightly concave, apically either rounded (Figs 90–92), or with blunt-angled projection bearing two subapical setae; inner margin slightly convex. Outer side of femur with regular or irregular row of 9–11 long, blunt setae and 2 subapical setae of same form (Figs 90–92). Inner-dorsal side of forefemur with few stout setae, length of these setae being half that of setae on dorsal side. Foreleg without patella-tibial suture, middle and hind legs with patella-tibial suture greatly shifted to apex of tibia. Posterior arm of U-shaped row of long setae on fore- and middle leg situated across tibia (Figs 90–91); on hind leg longitudinal (Fig. 92). Inner margin of tibia and tarsus with irregular, small, stout, pointed setae. Outer-apical seta of tibia blunt and elongate (Figs 90–92). Dorsal side of each tarsus with long, fine setae situated irregularly and partly forming two longitudinal rows. Claw either with two rows of denticles (Fig. 127) or with their vestiges (Fig. 126).

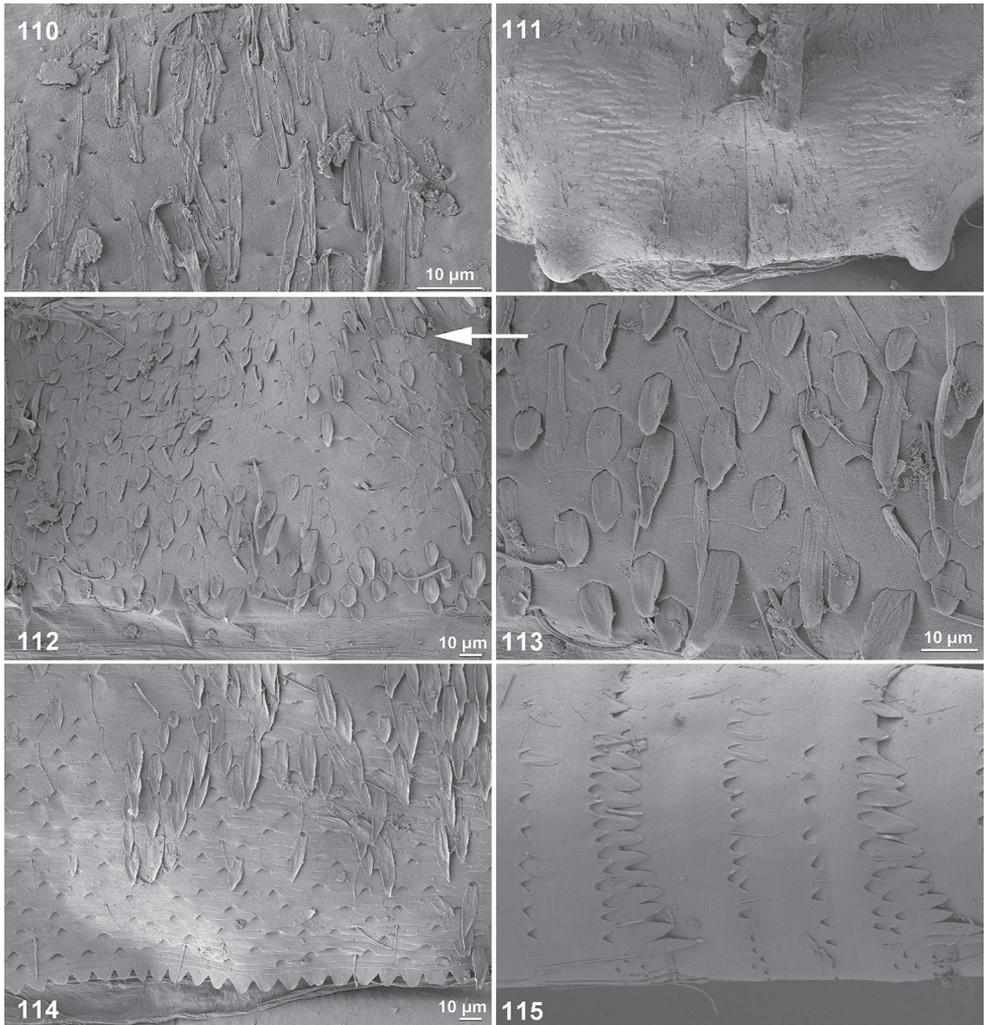
Scales on abdominal terga and sterna numerous, elongate, varying in size and shape, bordered by brown (Figs 94–122). Posterior margin of abdominal tergum I smooth, without denticles (Fig. 94); posterior margins of terga II–VI with short semicircular and triangular denticles (Figs 95–99); terga VII–IX with longer triangular denticles (Figs 100–102); on tergum IX middle part of hind margin behind pair of submedian setae lack denticles and projected posteriorly (Fig. 102). Posterior margin of tergum X without denticles on median part, laterally with paired groups of several



**Figures 90–93.** *Centropptella ingridae* sp. nov. (holotype). **90–92** tibia of fore, middle and hind leg, view from anterior side (bases of long setae shown both on anterior and posterior sides) **93** tenth abdominal segment without caudalii, ventral view.

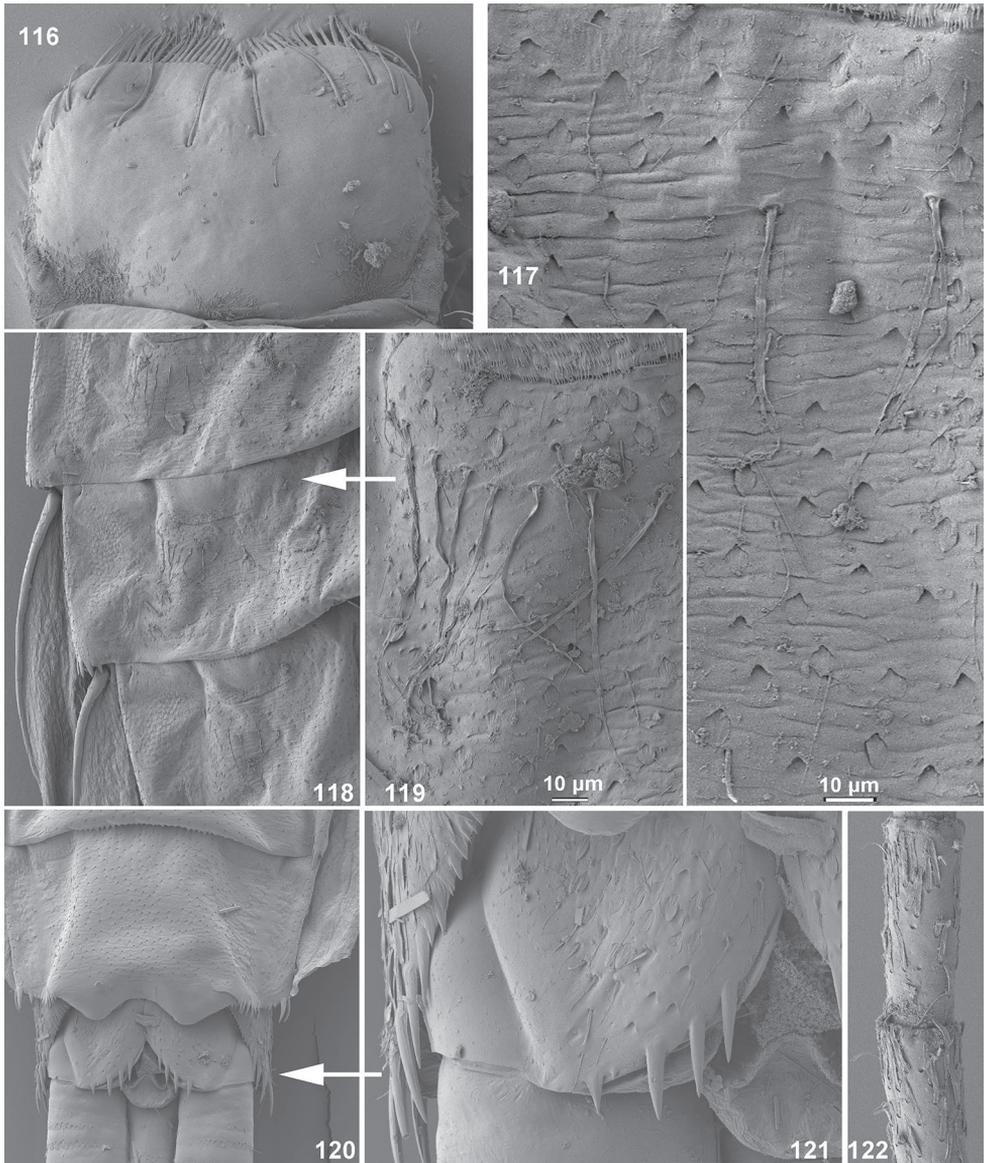


**Figures 94–109.** *Centroptella ingriddae* sp. nov. **94–102** fragments of abdominal terga I–IX (indicated by Roman numbers) **103–108** fragments of abdominal sterna IV–IX of male (indicated by Roman numbers) **109** fragment of abdominal sternum IX of female (**94–101** and **103–108** male paratype of *C. liebenauae*; **102** and **109** female specimen from Thailand).



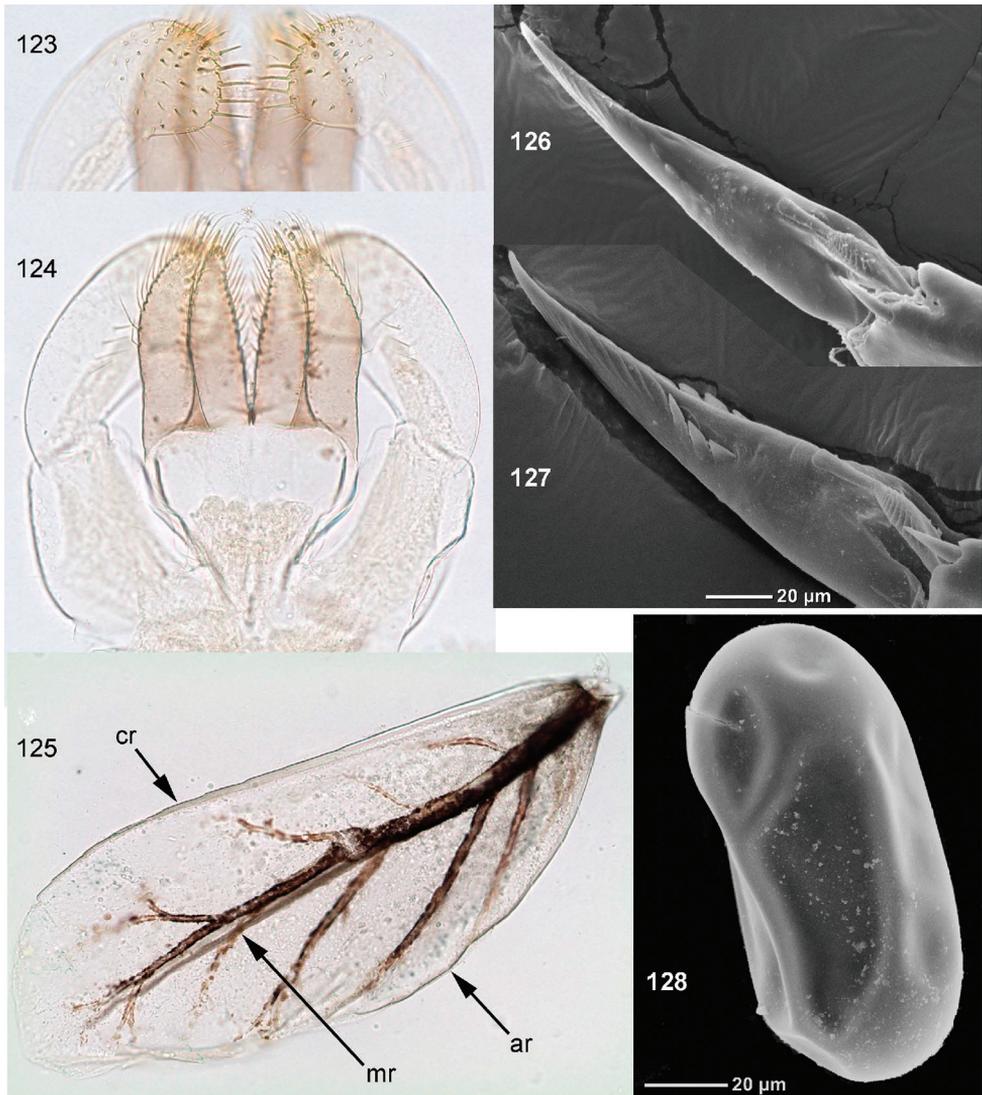
**Figures 110–115.** *Centropetella ingradae* sp. nov. (paratype of *C. liebenauae*), SEM photos of larva. **110** clypeus **111** pronotum **112–113** abdominal tergum I **114** abdominal tergum III **115** cercus.

denticles, decreasing in length in lateral direction (Fig. 93). Posterior margins of abdominal sterna I–IV smooth (Fig. 103); posterior margins of sterna V–VIII with regular, small, pointed, triangular denticles (Figs 104–107). Posterior margin of sternum IX in female convex, with row of triangular denticles diminished medially (Fig. 109), in male without denticles between protogonostyli, but with several denticles at sides (Figs 108, 148). Each sternum IV–VI with pair of regular, transverse rows of long, fine, bifurcate setae with spaced sockets (Figs 117–119); other sterna either without such setae, or with few, smaller setae irregularly situated. Paraprocts with small, an-



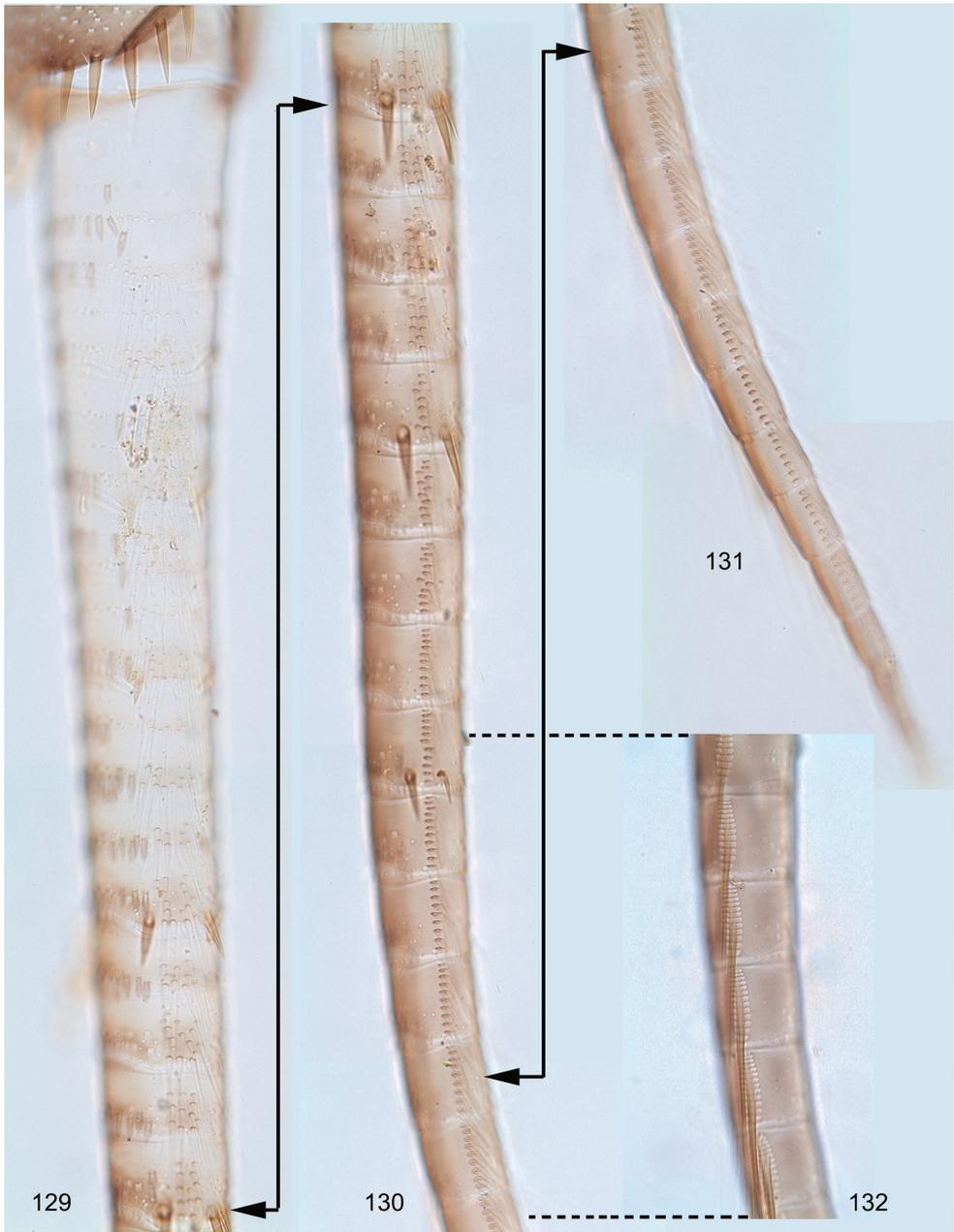
**Figures 116–122.** *Centroptella ingriddae* sp. nov. (paratype of *C. liebenauae*), SEM photos of larva. **116** labrum **117** bifurcate setae on abdominal sternum IV **118–119** abdominal sterna IV–V **120–121** paraprocts **122** flagellum of antenna.

terior, median apodeme, with few large pointed denticles on posterior margin, with scales as on sterna and terga (Figs 93, 120–121). Tergalius I narrow, elongate-ellipsoid; other tergalii wider, gradually changing in shape from tergaliius II to tergaliius VII (Figs 33–39). Each tergaliius II–VII, besides costal and anal ribs, with straight and narrow middle rib, located on dorsal surface on background of main trachea (Fig. 125). Costal



**Figures 123–128.** *Centropetella ingradae* sp. nov. (specimens from Thailand). **123–124** labium **125** tergalium IV (**cr** costal rib; **mr** middle rib; **ar** anal rib) **126–127** larval claws; **128** egg.

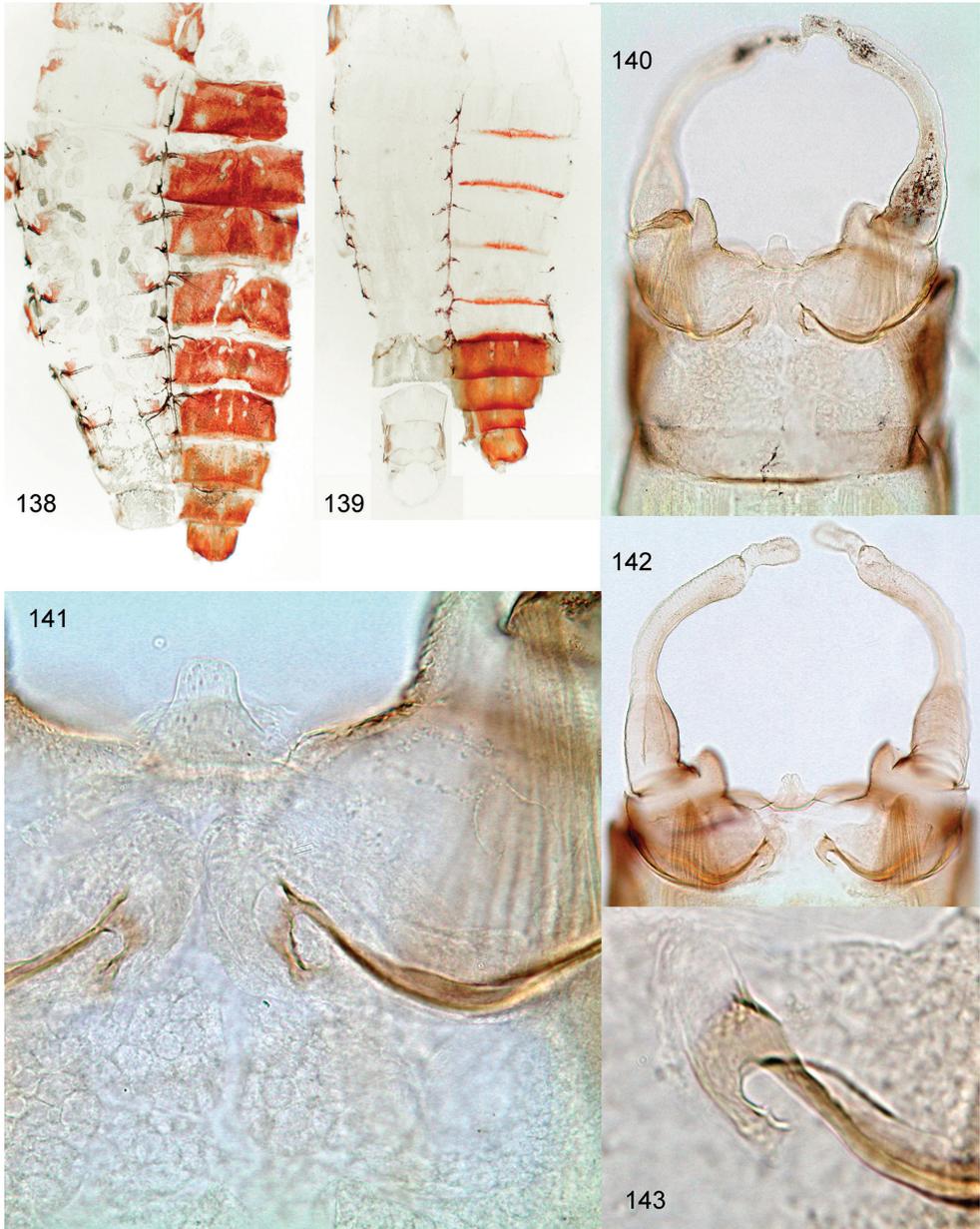
margin with poorly expressed serration; anal margin without serration; outer margin free of ribs, slightly notched, with small seta in each notch. In middle part of cercus, lateral side with 2 long, pointed denticles on every 4<sup>th</sup> segment (Figs 129–130). Each cercus, besides regular row of primary swimming setae on inner side (Fig. 132), with smaller and thinner secondary swimming setae on outer margin; on distal half of cercus, secondary swimming setae with wide, transverse, oval bases, forming regular row (Figs 129–131); on proximal half of cercus, secondary swimming setae with small, round bases and situated irregularly (Figs 129–130).



**Figures 129–132.** *Centroptella ingridae* sp. nov. (specimen from Thailand), exuviae of larval cercus (lateral view). **129–131** focus on lateral side to show bases of secondary swimming setae; **132** focus on median side to show bases of primary swimming setae.

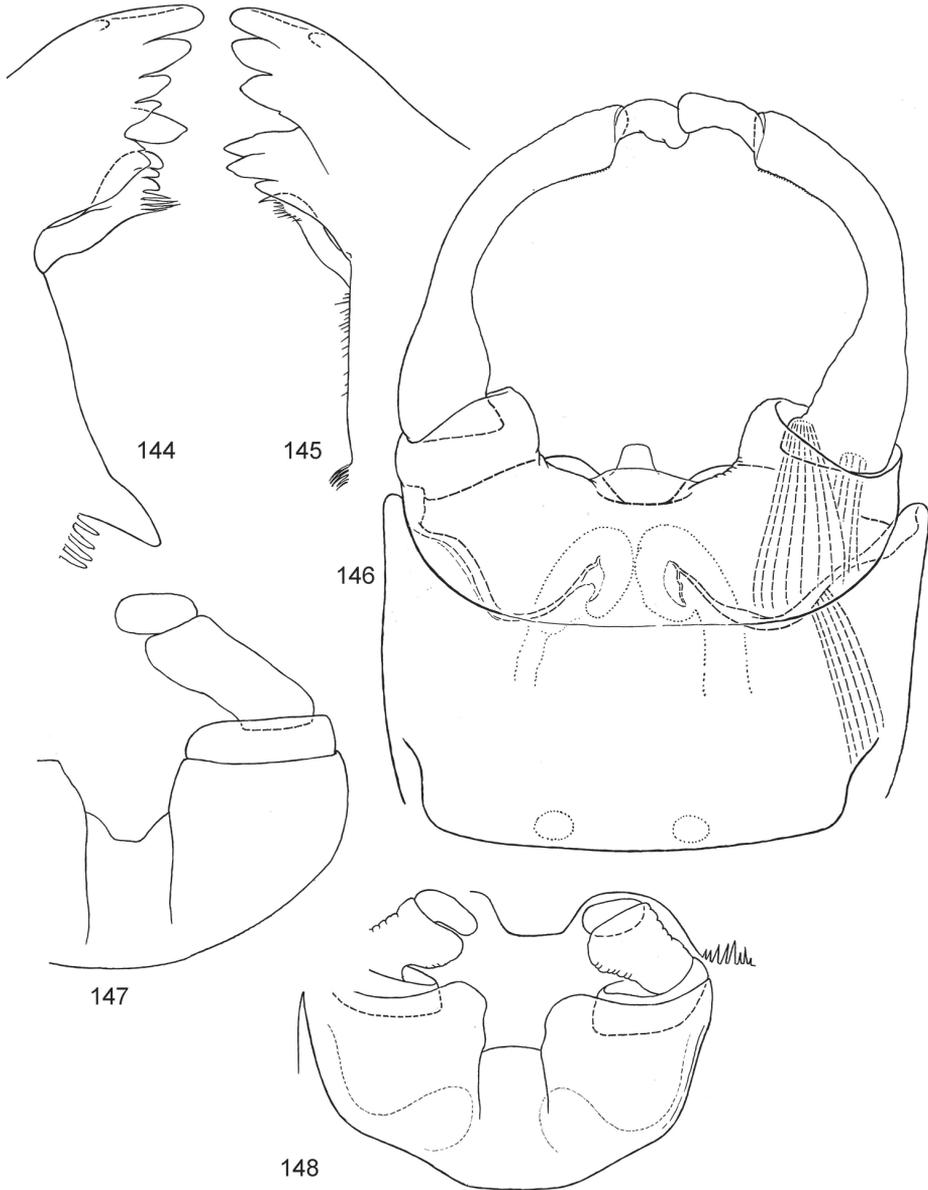


**Figures 133–137.** *Centropetella ingridae* sp. nov. (specimens from Thailand). **133–134** female imago **135–137** holotype (male): (**135**) head and thorax of imago (**136**) imaginal middle leg (**137**) subimaginal exuviae of tarsus.



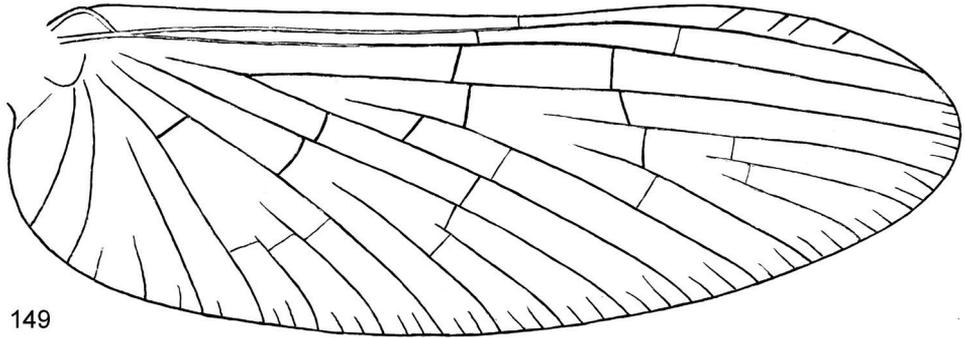
**Figures 138–143.** *Centroptella ingridae* sp. nov. (specimens from Thailand). **138** abdominal sterna and terga of female imago **139** the same, male imago **140–143** genitalia of male imago (**139–141** holotype).

*Male genitalia.* In last larval instar, developing subimaginal gonostyli folded under larval cuticle in peculiar pose, with 3<sup>rd</sup> segments bent medially-proximally (Fig. 148).



**Figures 144–148.** *Centroptella ingridae* sp. nov. (specimens from Thailand). **144–145** left and right mandibles **146** genitalia of male imago **147** their subimaginal exuviae **148** subimaginal gonostyli crumpled under larval cuticle (146–147 holotype).

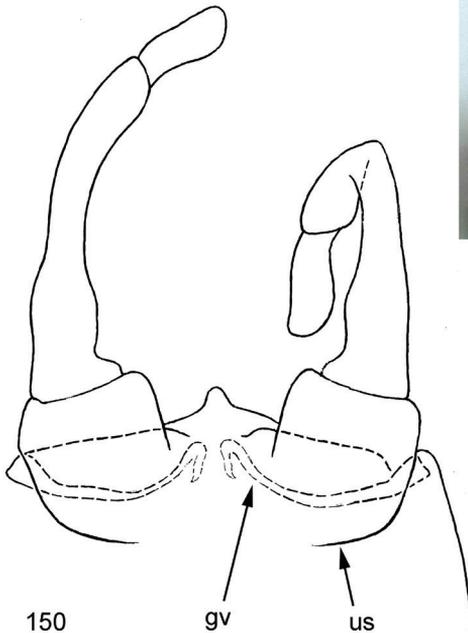
**Subimago.** Cuticle light brown with darker brown sutures of thorax; hypodermal coloration as in imago. On all legs of male and female all tarsal segments entirely covered by pointed microlepidies (Fig. 137).



149



151



150

gv

us



152

**Figures 149–152.** *Centroptella* of group *inzingae-ingridae*. *Centroptella ingridae* sp. nov. (holotype), fore wing **150** *Centroptella inzingae* (lectotype), genitalia (**gv** gonovectis **us** proximal margin of unistyliger) **151–152** *Centroptella saxophila*, gonovectes (specimens reared from larvae by N. Kluge in the Western Cape Province of South Africa).

**Imago, male.** Head brown. Turbinate eyes relatively low and wide, with yellow stem and orange-red faceted surface (Fig. 135). Thorax dark brown, with ochre pleural membranes (Fig. 135). Wing (Fig. 149) with membrane colourless, veins pale ochre or colourless, extreme base of costal and subcostal veins proximad of costal brace brown. Femora of all legs ochre, apically diffusely tinged with reddish; foretibia light

ochre, apically darkened with light brownish; middle and hind tibiae ochre, with diffuse longitudinal stripe; tarsi of all legs pale ochre; claws brown (Fig. 136). In holotype, length of femur, tibia and tarsal segments (mm) on foreleg 1.05 : 1.13 : 0.05 : 0.55 : 0.35 : 0.19 : 0.15, on middle leg 0.78 : 0.62 : 0.25 : 0.09 : 0.05 : 0.14, on hind leg 0.74 : 0.57 : 0.21 : 0.07 : 0.04 : 0.14. Tarsus of middle and hind leg with 1 apical spine on initial 3<sup>rd</sup> tarsomere (next after 1<sup>st</sup>+2<sup>nd</sup> tarsomere) (as in Fig 137). Abdominal tergum I colourless; terga II–VI colourless with narrow, contrasting, reddish stripe bordering posterior margin; terga VII–X red with ochre, with darker stripe bordering posterior margin; abdominal sterna colourless (Fig. 139). Genitalia (Figs 140–143, 146). Sterno-styligeral muscle entirely absent. Posterior margin of 9<sup>th</sup> abdominal sternum between unistyligers with narrow, trapezoid, membranous, colourless process (Figs 141, 146). Gonostylus with 1<sup>st</sup> segment narrowed apically; 2<sup>nd</sup> segment thickened toward apex; 3<sup>rd</sup> segment elongate, narrow and thickened toward apex (Figs 142, 146). Penial bridge medially sharply concave (Fig. 146). Gonovectes apically with sclerotized widenings of peculiar halberd-like shape (Figs 141, 143).

**Imago, female.** Head and thorax ochre with reddish markings (Figs 133–134). Leg coloration as in male. Patella-tibial suture present on middle and hind legs, absent on forelegs (as in male). Tarsus of each leg with 1 apical spine on initial 3<sup>rd</sup> tarsomere (on foreleg – on tarsomere next after 2<sup>nd</sup> tarsomere, on middle and hind leg—on tarsomere next after 1<sup>st</sup>+2<sup>nd</sup> tarsomere) (as in Fig 137). Abdominal terga intensely coloured with ochre and reddish, partly repeating cuticular colour pattern of larva; abdominal sterna nearly colourless, sterna I–VI with pair of reddish maculae near antero-lateral corners (Fig. 138).

**Egg.** Oval; chorion smooth, without relief (Fig. 128).

**Dimension.** Forewing length of male 4.7 mm; of female 5.0 mm.

**Distribution.** Indochina: known from Thailand and Vietnam; recently reported from China (Yunnan, Guangxi, Guangdong) under the species name *C. liebenauae* (Shi and Tong 2019).

**Comparison.** *Centroptella ingridae* sp. nov. belongs to the *inzingae-ingridae* species group; male imagines of this group differ from all other *Centroptella* by halberd-like gonovectes and absence of the sterno-styligeral muscle (Fig. 146). The male imago of *Centroptella ingridae* sp. nov. differs from other members of the *inzingae-ingridae* group by abdominal coloration (Figs 139). The larva of *Centroptella ingridae* sp. nov. differs from all other *Centroptella* by the presence of a pair of projections on the pronotum (Fig. 111).

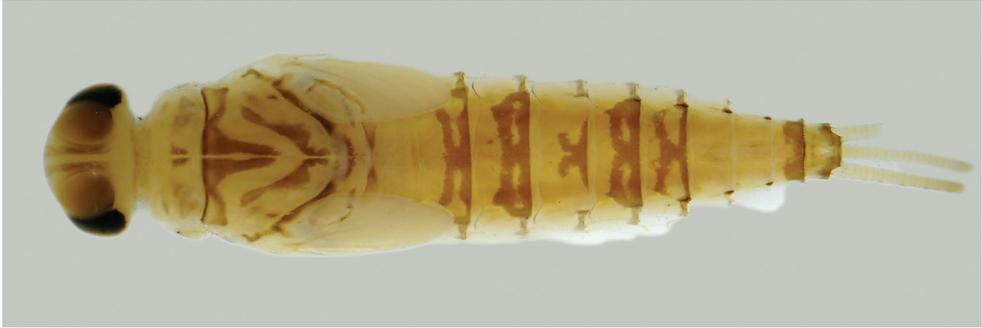
### ***Centroptella colorata* Soldán, Braasch & Muu, 1987**

Figure 153

*Centroptella colorata* Soldán, Braasch & Muu, 1987: 346 (larva)

*Chopralla colorata*: Tong and Dudgeon 2003: 17 (larval generic characters)

*Bungona (Chopralla) colorata*: Salles et al. 2016: 104; Shi and Tong 2019: 581



**Figure 153.** *Centroptella colorata* (holotype), last instar larva.

**Material examined. Holotype:** male larva of last instar, without caudalii, with labels: “VIETNAM, Lam Dong Prov, Da Nhim riv., Duc Trong, 27.X.1984, T. Soldán”, “*Centroptella colorata* T. Soldán det. 1985” and “HOLOTYPE”. Paratypes not found.

**Additional characters.** Abdominal tergum IV without denticles on posterior margin, so regular row of denticles present on posterior margin of terga V–IX only. Tergum X without denticles on median part of posterior margin, with one pair of large denticles at sides (as in *C. ceylonensis*).

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# Comprehensive approaches reveal three cryptic species of genus *Nidirana* (Anura, Ranidae) from China

Zhi-Tong Lyu<sup>1</sup>, Ke-Yuan Dai<sup>2</sup>, Yao Li<sup>1</sup>, Han Wan<sup>1</sup>, Zhe-Yi Liu<sup>1</sup>, Shuo Qi<sup>1,3</sup>,  
Si-Min Lin<sup>4</sup>, Jian Wang<sup>1</sup>, Yu-Long Li<sup>1</sup>, Yang-Jin Zeng<sup>2</sup>, Pi-Peng Li<sup>3</sup>,  
Hong Pang<sup>1</sup>, Ying-Yong Wang<sup>1</sup>

**1** State Key Laboratory of Biocontrol/ The Museum of Biology, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China **2** Guangdong Shimentai National Nature Reserve, Qingyuan 513000, China **3** Institute of herpetology, Shenyang Normal University, Shenyang 110034, China **4** Department of Life Science, National Taiwan Normal University, Taipei 116, China

Corresponding author: Ying-Yong Wang ([wangyy@mail.sysu.edu.cn](mailto:wangyy@mail.sysu.edu.cn)), Hong Pang ([lshpang@mail.sysu.edu.cn](mailto:lshpang@mail.sysu.edu.cn))

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## Abstract

Three cryptic species, which were previously reported as *Nidirana adenopleura*, are revealed on the basis of comprehensive approaches. *Nidirana guangdongensis* Lyu, Wan, and YY Wang, **sp. nov.** is distributed in Nanling Mountains and southern Luoxiao Mountains, *Nidirana mangveni* Lyu, Qi, and YY Wang, **sp. nov.** is known from northern Zhejiang, and *Nidirana xiangica* Lyu and YY Wang, **sp. nov.** occurs in Xiangjiang River Basin, while the true *Nidirana adenopleura* is designated from Taiwan Island, northern Fujian, southern Zhejiang, and central Jiangxi. These three new species can be distinguished from all congeners by significant divergences in the mitochondrial 16S and CO1 genes, differences in advertisement calls, and the combination of multiple characteristics. This work indicates that the current records of *Nidirana adenopleura* should be of a species complex composed of multiple species and have clarified the true identity of *N. adenopleura*.

## Keywords

bioacoustics, mitochondrial DNA, morphology, *Nidirana guangdongensis* sp. nov., *Nidirana mangveni* sp. nov., *Nidirana xiangica* sp. nov.

## Introduction

The Music frog genus *Nidirana* Dubois, 1992 was recently reconsidered as a distinct genus based on comprehensive approaches (Lyu et al. 2017). Ten species are currently recognized from subtropical eastern and southeastern Asia: *N. okinavana* (Boettger, 1895) from Yaeyama of southern Ryukyu, and eastern Taiwan; *N. adenopleura* (Boulenger, 1909) from Taiwan and southeastern mainland China; *N. nankunensis* Lyu, Zeng, Wang, Lin, Liu, & Wang, 2017 from Mt Nankun of Guangdong; *N. yaoica* Lyu, Mo, Wan, Li, Pang, & Wang, 2019 from Mt Dayao of Guangxi; *N. hainanensis* (Fei, Ye, & Jiang, 2007) from Mt Diaoluo of Hainan; *N. leishanensis* Li, Wei, Xu, Cui, Fei, Jiang, Liu, & Wang, 2019 from Mt Leigong of Guizhou; *N. daunchina* (Chang, 1933) from western China; *N. pleuraden* (Boulenger, 1904) from southwestern China; and *N. chapaensis* (Bourret, 1937) and *N. lini* (Chou, 1999) from the northeastern Indochinese peninsula.

Among the species in genus *Nidirana*, *N. adenopleura* has the widest distribution area and has been reported from Taiwan, Fujian, Zhejiang, Anhui, Jiangxi, Guangdong, Guangxi, Hunan and Guizhou (Fei et al. 2009, 2012). In the previous study (Lyu et al. 2017), the populations from Taiwan, northern Fujian, Jingning County of Zhejiang, and Mt Jinggang of Jiangxi were confirmed as the same species, which also synonymized *N. caldwelli* Schmidt, 1925 with *N. adenopleura*. Besides, it is worth noting that the frogs previously considered as *N. adenopleura* from Mt Dayao of Guangxi and Mt Leigong of Guizhou were respectively revealed as two new species, *N. yaoica* and *N. leishanensis*, most recently (Lyu et al. 2019; Li et al. 2019). Nevertheless, the exact taxonomic statuses of other *N. adenopleura* populations from China have not yet been tested.

Through our herpetological surveys throughout southeastern China, we have collected a series of *Nidirana* specimens which were previously reported as *N. adenopleura* (Fei et al. 2009, 2012; Li et al. 2011; Mo et al. 2014). However, comprehensive analyses of molecules, bioacoustics, and morphology have indicated that these specimens are distinctive from all known congeners including the true *N. adenopleura* (designated here as *N. adenopleura sensu stricto*), which suggests they should belong to three unnamed cryptic species. Therefore, based on the results of our present work, we herein describe them as three new species of the genus *Nidirana*.

## Materials and methods

### Taxon sampling

For the molecular analysis, a total of 54 muscular samples of *Nidirana* were used, of which 41 are from the undescribed specimens, eight from the true *N. adenopleura*, two from *N. hainanensis* and three from *N. leishanensis*. All samples were attained from euthanized specimens and then preserved in 95% ethanol and stored at -40 °C. In ad-

dition, 43 sequences from all known *Nidirana* congeners and two sequences from the out-group *Babina* Thompson, 1912 (following Lyu et al. 2017) were obtained from GenBank and incorporated into our dataset. Detailed information of these materials is shown in Table 1 and Fig. 1.

### DNA Extraction, PCR amplification, and sequencing

Genomic DNA were extracted from muscle tissue samples, using DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. Two mitochondrion genes, namely partial 16S ribosomal RNA gene (16S) and partial cytochrome C oxidase 1 gene (CO1), were amplified. Primers used for 16S were L3975 (5'-CGCCTGTTTACCAAAAACAT-3') and H4551 (5'-CCGGTCTGAACTCAGATCACGT-3'), and L2A (5'-CCAAACGAGCCTAGTGATAGCTGGTT-3') and H10 (5'-TGATTACGCTACCTTTGCACGGT-3'), and for CO1 were dgLCO (5'-GGTCAACAAATCATAAAGAYATYGG-3') and dgHCO (5'-AAACTTCAGGGTGACCAARAAYCA-3'), following Lyu et al. (2019). PCR amplifications were processed with the cycling conditions that initial denaturing step at 95°C for 4 min, 35 cycles of denaturing at 94°C for 40 s, annealing at 53°C (for 16S) / 48 °C (for CO1) for 40 s and extending at 72°C for 60 s, and a final extending step at 72°C for 10 min. PCR products were purified with spin columns and then sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit per the guidelines, on an ABI Prism 3730 automated DNA sequencer by Shanghai Majorbio Bio-pharm Technology Co, Ltd. All sequences were deposited in GenBank (Table 1).

### Phylogenetic analyses

DNA sequences were aligned by the Clustal W algorithm with default parameters (Thompson et al. 1997) and trimmed with the gaps partially deleted in MEGA 6 (Tamura et al. 2013). Two gene segments, 644 base pairs (bp) of CO1 and 1049 bp of 16S, were concatenated seriatim into a 1693-bp sequence, and were further tested in jmodeltest v2.1.2 with Akaike and Bayesian information criteria, all resulting the best-fitting nucleotide substitution models of GTR+I+G. Sequenced data was analyzed using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012), and maximum likelihood (ML) in RaxmlGUI 1.3 (Silvestro and Michalak 2012). Two independent runs were conducted in a BI analysis, each of which was performed for 10,000,000 generations and sampled every 1000 generations with the first 25% samples were discarded as burn-in, resulting a potential scale reduction factor (PSRF) of < 0.005. In ML analysis, the bootstrap consensus tree inferred from 1000 replicates was used to represent the evolutionary history of the taxa analyzed. Mean genetic distances between and within species were calculated in MEGA 6 using the uncorrected *p*-distance model.

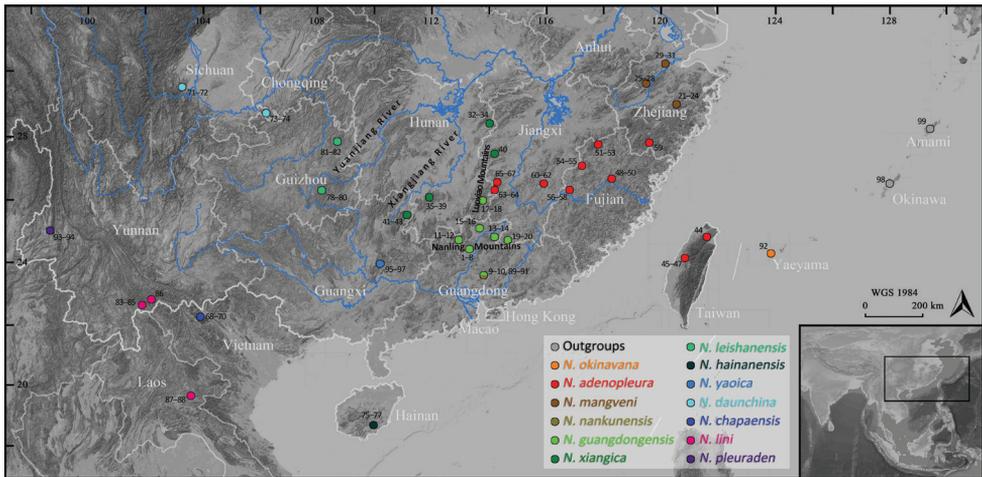
**Table 1.** Localities, voucher information, and GenBank numbers for all samples used in this study. An asterisk denotes type localities.

ID	Species	Localities	Voucher number	16S	CO1
1	<i>Nidirana guangdongensis</i>	China: Guangdong: Shimentai Nature Reserve *	SYS a005765	MN946404	MN945160
2	<i>Nidirana guangdongensis</i>	China: Guangdong: Shimentai Nature Reserve *	SYS a005766	MN946405	MN945161
3	<i>Nidirana guangdongensis</i>	China: Guangdong: Shimentai Nature Reserve *	SYS a005767	MN946406	MN945162
4	<i>Nidirana guangdongensis</i>	China: Guangdong: Shimentai Nature Reserve *	SYS a005768	MN946407	MN945163
5	<i>Nidirana guangdongensis</i>	China: Guangdong: Shimentai Nature Reserve *	SYS a005995	MN946408	MN945164
6	<i>Nidirana guangdongensis</i>	China: Guangdong: Shimentai Nature Reserve *	SYS a005996	MN946409	MN945165
7	<i>Nidirana guangdongensis</i>	China: Guangdong: Shimentai Nature Reserve *	SYS a005997	MN946410	MN945166
8	<i>Nidirana guangdongensis</i>	China: Guangdong: Shimentai Nature Reserve *	SYS a005998	MN946411	MN945167
9	<i>Nidirana guangdongensis</i>	China: Guangdong: Mt Nankun	SYS a005720	MN946412	MN945168
10	<i>Nidirana guangdongensis</i>	China: Guangdong: Mt Nankun	SYS a005721	MN946413	MN945169
11	<i>Nidirana guangdongensis</i>	China: Guangdong: Mt Tianjing	SYS a006934	MN946414	MN945170
12	<i>Nidirana guangdongensis</i>	China: Guangdong: Mt Tianjing	SYS a006935	MN946415	MN945171
13	<i>Nidirana guangdongensis</i>	China: Guangdong: Mt Chebaling	SYS a007900	MN946416	MN945172
14	<i>Nidirana guangdongensis</i>	China: Guangdong: Mt Chebaling	SYS a007901	MN946417	MN945173
15	<i>Nidirana guangdongensis</i>	China: Guangdong: Renhua County	SYS a008135	MN946418	MN945174
16	<i>Nidirana guangdongensis</i>	China: Guangdong: Renhua County	SYS a008136	MN946419	MN945175
17	<i>Nidirana guangdongensis</i>	China: Hunan: Mt Bamian	SYS a006195	MN946420	MN945176
18	<i>Nidirana guangdongensis</i>	China: Hunan: Mt Bamian	SYS a006196	MN946421	MN945177
19	<i>Nidirana guangdongensis</i>	China: Jiangxi: Mt Jiulian	SYS a004071	MN946422	MN945178
20	<i>Nidirana guangdongensis</i>	China: Jiangxi: Mt Jiulian	SYS a004082	MN946423	MN945179
21	<i>Nidirana mangveni</i>	China: Zhejiang: Mt Dapan *	SYS a006310	MN946424	MN945180
22	<i>Nidirana mangveni</i>	China: Zhejiang: Mt Dapan *	SYS a006311	MN946425	MN945181
23	<i>Nidirana mangveni</i>	China: Zhejiang: Mt Dapan *	SYS a006312	MN946426	MN945182
24	<i>Nidirana mangveni</i>	China: Zhejiang: Mt Dapan *	SYS a006313	MN946427	MN945183
25	<i>Nidirana mangveni</i>	China: Zhejiang: Mt Longmen	SYS a006413	MN946428	MN945184
26	<i>Nidirana mangveni</i>	China: Zhejiang: Mt Longmen	SYS a006414	MN946429	MN945185
27	<i>Nidirana mangveni</i>	China: Zhejiang: Mt Longmen	SYS a006415	MN946430	MN945186
28	<i>Nidirana mangveni</i>	China: Zhejiang: Mt Longmen	SYS a006416	MN946431	MN945187
29	<i>Nidirana mangveni</i>	China: Zhejiang: Hangzhou City	SYS a007990	MN946432	MN945188
30	<i>Nidirana mangveni</i>	China: Zhejiang: Hangzhou City	SYNU12050567	KF020600	KF020615
31	<i>Nidirana mangveni</i>	China: Zhejiang: Hangzhou City	SYNU12050568	KF020601	KF020616
32	<i>Nidirana xiangica</i>	China: Hunan: Mt Dawei *	SYS a006491	MN946433	MN945189
33	<i>Nidirana xiangica</i>	China: Hunan: Mt Dawei *	SYS a006492	MN946434	MN945190
34	<i>Nidirana xiangica</i>	China: Hunan: Mt Dawei *	SYS a006493	MN946435	MN945191
35	<i>Nidirana xiangica</i>	China: Hunan: Mt Yangming	SYS a007269	MN946436	MN945192
36	<i>Nidirana xiangica</i>	China: Hunan: Mt Yangming	SYS a007270	MN946437	MN945193
37	<i>Nidirana xiangica</i>	China: Hunan: Mt Yangming	SYS a007271	MN946438	MN945194
38	<i>Nidirana xiangica</i>	China: Hunan: Mt Yangming	SYS a007272	MN946439	MN945195
39	<i>Nidirana xiangica</i>	China: Hunan: Mt Yangming	SYS a007273	MN946440	MN945196
40	<i>Nidirana xiangica</i>	China: Jiangxi: Mt Wugong	SYS a002590	MN946441	MN945197
41	<i>Nidirana xiangica</i>	China: Guangxi: Mt Dupangling	SYS a006568	MN946442	MN945198
42	<i>Nidirana xiangica</i>	China: Guangxi: Mt Dupangling	SYS a006569	MN946443	MN945199
43	<i>Nidirana xiangica</i>	China: Guangxi: Mt Dupangling	SYS a006570	MN946444	MN945200
44	<i>Nidirana adenopleura</i>	China: Taiwan: New Taipei City	UMMZ 189963	DQ283117	/
45	<i>Nidirana adenopleura</i>	China: Taiwan: Taichung City	SYS a007358	MN946445	MN945201
46	<i>Nidirana adenopleura</i>	China: Taiwan: Taichung City	SYS a007359	MN946446	MN945202
47	<i>Nidirana adenopleura</i>	China: Taiwan: Taichung City	SYS a007360	MN946447	MN945203
48	<i>Nidirana adenopleura</i>	China: Fujian: Nanping City	SYS a005911	MF807844	MF807883
49	<i>Nidirana adenopleura</i>	China: Fujian: Nanping City	SYS a005912	MF807845	MF807884
50	<i>Nidirana adenopleura</i>	China: Fujian: Nanping City	SYS a005913	MF807846	MF807885
51	<i>Nidirana adenopleura</i>	China: Fujian: Mt Wuyi	SYS a005939	MF807850	MF807889
52	<i>Nidirana adenopleura</i>	China: Fujian: Mt Wuyi	SYS a005940	MF807851	MF807890
53	<i>Nidirana adenopleura</i>	China: Fujian: Mt Wuyi	SYS a005941	MF807852	MF807891
54	<i>Nidirana adenopleura</i>	China: Fujian: Jiangshi Nature Reserve	SYS a004112	MF807833	MF807872

ID	Species	Localities	Voucher number	16S	CO1
55	<i>Nidirana adenopleura</i>	China: Fujian: Jiangshi Nature Reserve	SYS a004132	MF807834	MF807873
56	<i>Nidirana adenopleura</i>	China: Fujian: Mt Yashu	SYS a005891	MF807841	MF807880
57	<i>Nidirana adenopleura</i>	China: Fujian: Mt Yashu	SYS a005901	MF807842	MF807881
58	<i>Nidirana adenopleura</i>	China: Fujian: Mt Yashu	SYS a005902	MF807843	MF807882
59	<i>Nidirana adenopleura</i>	China: Zhejiang: Jingning County	SYS a002725	MF807827	MF807866
60	<i>Nidirana adenopleura</i>	China: Jiangxi: Ningdu County	SYS a007089	MN946448	MN945204
61	<i>Nidirana adenopleura</i>	China: Jiangxi: Ningdu County	SYS a007090	MN946449	MN945205
62	<i>Nidirana adenopleura</i>	China: Jiangxi: Ningdu County	SYS a007091	MN946450	MN945206
63	<i>Nidirana adenopleura</i>	China: Jiangxi: Shuichuang County	SYS a004450	MN946456	MN945212
64	<i>Nidirana adenopleura</i>	China: Jiangxi: Shuichuang County	SYS a004451	MN946457	MN945213
65	<i>Nidirana adenopleura</i>	China: Jiangxi: Jinggangshan Nature Reserve	SYS a004025	MF807830	MF807869
66	<i>Nidirana adenopleura</i>	China: Jiangxi: Jinggangshan Nature Reserve	SYS a004026	MF807831	MF807870
67	<i>Nidirana adenopleura</i>	China: Jiangxi: Jinggangshan Nature Reserve	SYS a004027	MF807832	MF807871
68	<i>Nidirana chapaensis</i>	Vietnam: Lao Cai: Sapa *	T2483/2000.4850	KR827711	KR087625
69	<i>Nidirana chapaensis</i>	Vietnam: Lao Cai: Sapa *	1999.5871	KR827710	/
70	<i>Nidirana chapaensis</i>	Vietnam: Lao Cai: Sapa *	ROM 28070	AF206460	/
71	<i>Nidirana daunchina</i>	China: Sichuan: Mt Emei *	SYS a004594	MF807822	MF807861
72	<i>Nidirana daunchina</i>	China: Sichuan: Mt Emei *	SYS a004595	MF807823	MF807862
73	<i>Nidirana daunchina</i>	China: Sichuan: Hejiang County	SYS a004930	MF807824	MF807863
74	<i>Nidirana daunchina</i>	China: Sichuan: Hejiang County	SYS a004931	MF807825	MF807864
75	<i>Nidirana hainanensis</i>	China: Hainan: Mt Diaoluo	SYS a003741	MF807821	MF807860
76	<i>Nidirana hainanensis</i>	China: Hainan: Mt Diaoluo	SYS a007669	MN946451	MN945207
77	<i>Nidirana hainanensis</i>	China: Hainan: Mt Diaoluo	SYS a007670	MN946452	MN945208
78	<i>Nidirana leishanensis</i>	China: Guizhou: Mt Leigong *	CIBLS20150627003	MK293810	MK293828
79	<i>Nidirana leishanensis</i>	China: Guizhou: Mt Leigong *	CIBLS20150628002	MK293812	MK293830
80	<i>Nidirana leishanensis</i>	China: Guizhou: Mt Leigong *	SYS a007908	MN946453	MN945209
81	<i>Nidirana leishanensis</i>	China: Guizhou: Mt Fanjing	SYS a007195	MN946454	MN945210
82	<i>Nidirana leishanensis</i>	China: Guizhou: Mt Fanjing	SYS a007196	MN946455	MN945211
83	<i>Nidirana lini</i>	China: Yunnan: Jiangcheng County *	SYS a003967	MF807818	MF807857
84	<i>Nidirana lini</i>	China: Yunnan: Jiangcheng County *	SYS a003968	MF807819	MF807858
85	<i>Nidirana lini</i>	China: Yunnan: Jiangcheng County *	SYS a003969	MF807820	MF807859
86	<i>Nidirana lini</i>	China: Yunnan: Lyuchun County	HNNULC001	KF185066	/
87	<i>Nidirana lini</i>	Laos: Xieng Khouang	FMNH256531	KR264073	/
88	<i>Nidirana lini</i>	Laos: Xieng Khouang	FMNH256532	KR264074	/
89	<i>Nidirana nankunensis</i>	China: Guangdong: Mt Nankun *	SYS a005717	MF807838	MF807877
90	<i>Nidirana nankunensis</i>	China: Guangdong: Mt Nankun *	SYS a005718	MF807839	MF807878
91	<i>Nidirana nankunensis</i>	China: Guangdong: Mt Nankun *	SYS a005719	MF807840	MF807879
92	<i>Nidirana okinavana</i>	Japan: Okinawa: Iriomote Island *	Not given	NC022872	NC022872
93	<i>Nidirana pleuraden</i>	China: Yunnan: Mt Gaoligong	SYS a003775	MF807816	MF807855
94	<i>Nidirana pleuraden</i>	China: Yunnan: Mt Gaoligong	SYS a003776	MF807817	MF807856
95	<i>Nidirana yaoica</i>	China: Guangxi: Mt Dayao *	SYS a007020	MK882276	MK895041
96	<i>Nidirana yaoica</i>	China: Guangxi: Mt Dayao *	SYS a007021	MK882277	MK895042
97	<i>Nidirana yaoica</i>	China: Guangxi: Mt Dayao *	SYS a007022	MK882278	MK895043
98	<i>Babina bolsti</i>	Japan: Okinawa *	Not given	NC022870	NC022870
99	<i>Babina subaspera</i>	Japan: Kagoshima: Amami Island *	Not given	NC022871	NC022871

## Bioacoustics analysis

Advertisement calls were recorded in the field at the air temperature of 18–20 °C using a SONY PCM D100 digital sound recorder. The sound files in wave format were sampled at 44.1 kHz with 24 bits in depth. Praat 6.0.27 (Boersma 2001) was used to obtain the oscillogram, sonogram, and power spectrum (window length = 0.005 s). Raven pro 1.5 (Cornell Lab of Ornithology, 2003–2014) was used to quantify the acoustic properties (window size = 1024 points, fast Fourier transform, Hamming



**Figure 1.** Localities of the *Nidirana* and outgroup *Babina* samples used in this study. Numbers correspond to the ID numbers in Table 1.

window with no overlap). The following measurements were taken for each call: call duration (the time between onset of the first note and offset of the last note in a call), note duration (the time between onset and offset of a note), note rise time (the time between onset and max amplitude of a note), and note interval (the time between adjacent notes in a call). Comparison bioacoustics descriptions of known congeners were obtained from the literature (Matsui and Utsunomiya 1983; Chou 1999; Fei et al. 2007, 2009; Chuaynkern et al. 2010; Lyu et al. 2017, 2019; Li et al. 2019).

## Morphology

Comparison characters of all known congeners were obtained from the literature (Boettger 1895; Boulenger 1904, 1909; Schmidt 1925; Chang and Hsu 1932; Bourret 1937; Kuramoto 1985; Chou 1999; Fei et al. 2007, 2009; Matsui 2007; Chuaynkern et al. 2010; Lyu et al. 2017, 2019; Li et al. 2019) and 74 examined museum specimens of seven species which are listed in the Appendix. All specimens were fixed in 10% buffered formalin and later transferred to 70% ethanol, and deposited in the Museum of Biology, Sun Yat-sen University (SYS), Institute of Herpetology, Shenyang Normal University (SYNU), Natural History Museum of Guangxi (NHMG), and Chengdu Institute of Biology, Chinese Academy of Sciences (CIB), China.

Morphological descriptions mainly follow Fei et al. (2009), Chuaynkern et al. (2010) and Lyu et al. (2017). Sex and age were determined by secondary sexual characters, i.e., the presence of suprabrachial glands in males. Webbing formula was written according to Savage (1975). External measurements were made for the unnamed *Nidirana* specimens and 18 specimens of *N. adenopleura*, with digital calipers (Neiko

01407A Stainless Steel 6-Inch Digital Caliper, USA) to the nearest 0.1 mm. These measurements were as follows:

- SVL** snout-vent length (from tip of snout to posterior margin of vent);
- HDL** head length (from tip of snout to the articulation of the jaw);
- HDW** head width (head width at the commissure of the jaws);
- SNT** snout length (from tip of snout to the anterior corner of the eye);
- IND** internasal distance (distance between nares);
- IOD** interorbital distance (minimum distance between upper eyelids);
- ED** eye diameter (from the anterior corner of the eye to posterior corner of the eye);
- TD** tympanum diameter (horizontal diameter of tympanum);
- TED** tympanum-eye distance (from anterior edge of tympanum to posterior corner of the eye);
- HND** hand length (from the proximal border of the outer palmar tubercle to the tip of digit III);
- RAD** radio-ulna length (from the flexed elbow to the proximal border of the outer palmar tubercle);
- FTL** foot length (from distal end of shank to the tip of digit IV);
- TIB** tibial length (from the outer surface of the flexed knee to the heel).

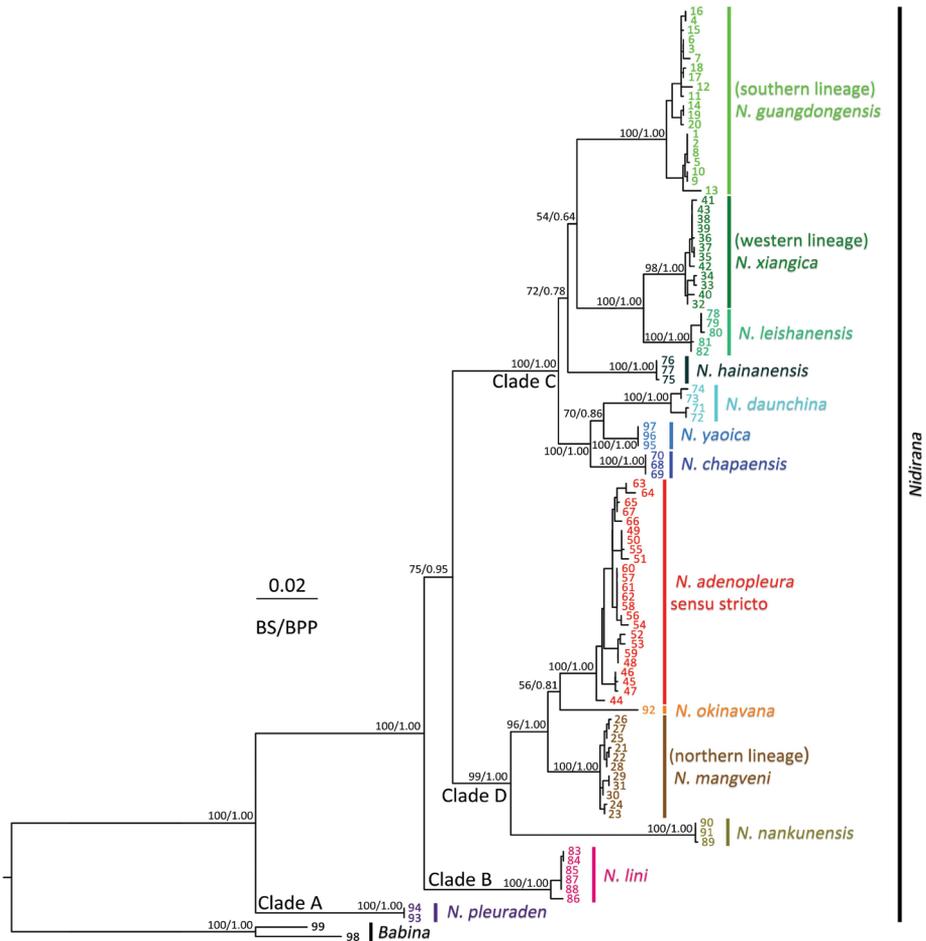
Principal component analysis (PCA), one-way analysis of variance (ANOVA) and Tukey test for multiple comparisons, were performed on the adult male specimens, of which the morphometric measurements were ln-transformed in order to normalize the variables, to test the significance of differences on morphometric characters among different species, using R 3.3.2 (R Core Team 2016).

## Results

### Phylogenetic analyses

The ML and BI analyses resulted in essentially identical topologies and were integrated in Fig. 2, in which the major nodes were sufficiently supported with the bootstrap supports (BS) for maximum likelihood analysis > 75 and the Bayesian posterior probabilities (BPP) > 0.95. Mean  $p$ -distance among all in-group and out-group species used in this study are given in Table 2.

In the phylogenetic result, all samples of genus *Nidirana* formed a monophyletic group, which can be further divided into four highly supported clades A, B, C, and D (the names of clades follow Lyu et al. (2017)). This result is consistent with the phylogenetic relationship in previous studies (Lyu et al. 2017, 2019; Li et al. 2019). However, the relationship among clades B, C, and D remains unresolved due to the insignificant supported values among these clades.



**Figure 2.** Phylogenetic tree based on mitochondrial 16S + CO1 genes. Numbers at tips of branches correspond to the ID numbers in Table 1.

Within clade D, the samples from Taiwan, northern Fujian, southern Zhejiang and central Jiangxi are grouped in a distinct and substantial single lineage (red color in Figs. 1 and 2) that represent the *Nidirana adenopleura* s. s. whose type locality is in Taiwan Island. Nevertheless, the samples from northern Zhejiang (brown color in Figs. 1 and 2), which were previously recorded as *N. adenopleura*, are grouped in a distinct single lineage (designated here as the northern lineage), that is non-monophyletic with the lineage of *N. adenopleura* s. s. and has significant divergences against all congeners. In addition, the samples from Xiangjiang River Basin (dark green color in Figs. 1 and 2) and from Nanling Mountains and southern Luoxiao Mountains (bright green color in Figs. 1 and 2), which were also previously considered as *N. adenopleura*, form two distinct lineages with significant divergences respectively (designated here as the western and southern lineages respectively) within clade C, which are both significantly distant from the lineage of the *N. adenopleura* s. s. in clade D.

**Table 2.** Mean *p*-distance gene among the *Nidirana* and *Babina* species used in this study.

ID	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	<i>Nidirana guangdongensis</i>	0.6														
2	<i>Nidirana mangveni</i>	7.1	0.3													
3	<i>Nidirana xiangica</i>	5.0	7.5	0.3												
4	<i>Nidirana adenopleura</i>	6.7	3.1	7.6	0.8											
5	<i>Nidirana chapaensis</i>	4.4	5.5	4.0	5.2	0.0										
6	<i>Nidirana daunchina</i>	5.0	7.1	5.1	6.7	3.0	0.6									
7	<i>Nidirana hainanensis</i>	4.6	6.9	4.5	6.8	3.7	5.1	0.0								
8	<i>Nidirana leishanensis</i>	5.1	7.3	2.4	6.9	4.4	5.2	4.4	0.2							
9	<i>Nidirana lini</i>	6.8	5.6	7.0	6.0	5.0	6.7	6.0	6.4	0.2						
10	<i>Nidirana nankunensis</i>	8.4	5.8	8.7	6.3	8.2	9.0	8.5	8.1	7.6	0.0					
11	<i>Nidirana okinavana</i>	7.2	3.4	8.2	3.4	5.5	7.3	7.3	7.6	6.6	6.1	/				
12	<i>Nidirana pleuraden</i>	9.9	8.5	10.2	8.9	7.8	9.2	9.4	10.4	7.8	10.3	9.3	0.0			
13	<i>Nidirana yaoica</i>	4.6	6.7	4.6	6.0	2.4	2.8	4.1	4.5	6.4	8.5	6.8	9.3	0.0		
14	<i>Babina holsti</i>	15.0	13.9	15.6	14.3	13.5	14.6	15.0	15.7	13.1	15.1	14.6	12.7	15.0	/	
15	<i>Babina subaspera</i>	14.9	13.8	15.4	14.2	13.0	14.5	14.8	15.2	12.9	14.7	14.6	12.7	15.0	3.3	/

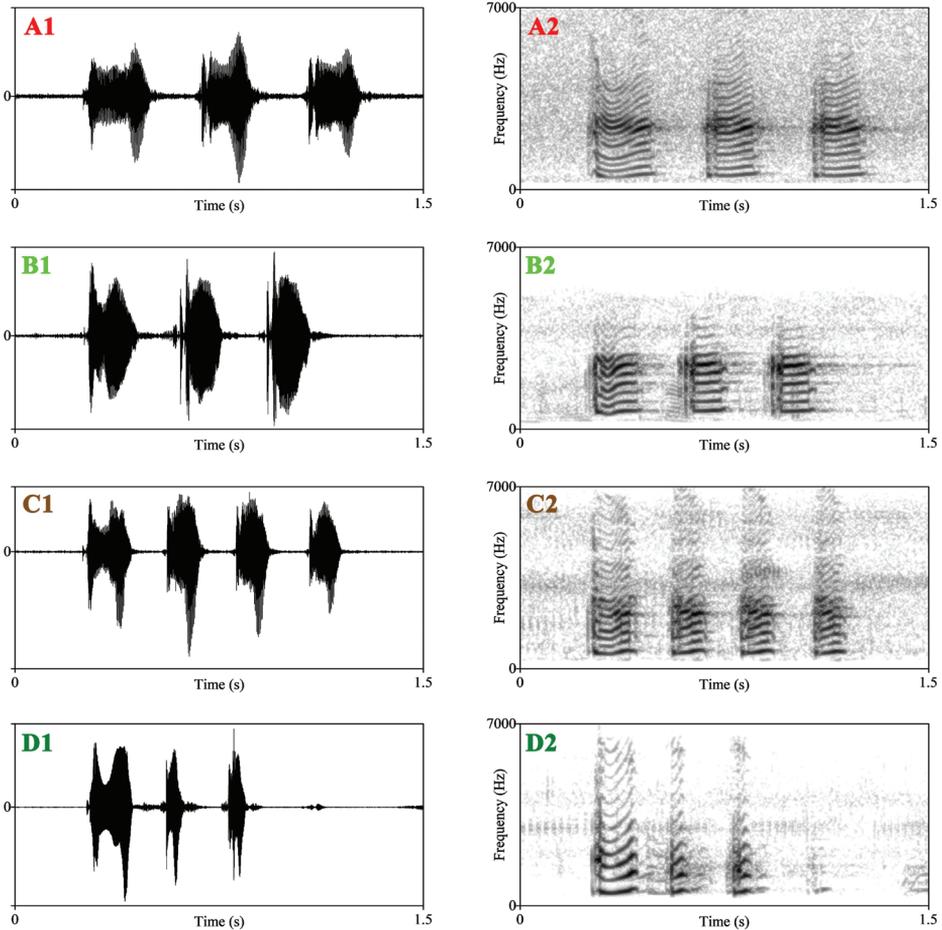
This phylogenetic result indicates that the previous identifications for the populations from northern Zhejiang (northern lineage), from Xiangjiang River Basin (western lineage), and from Nanling Mountains and southern Luoxiao Mountains (southern lineage) are incorrect, and these three populations represent three separate evolutionary lineages within the genus *Nidirana*.

### Bioacoustics analysis

The call spectrograms of *Nidirana adenopleura* s. s. and the three unnamed lineages are shown in Fig. 3 and the measurement parameters are listed in Table 3.

The advertisement calls of the southern lineage is different from the congeners by (1) containing 2–4 ( $2.9 \pm 0.7$ ,  $N = 54$ ) identical regular notes vs. containing 10–25 fast-repeated regular notes in *Nidirana okinavana*; containing 5–7 regular notes in *N. lini*; containing 4–7 regular notes in *N. pleuraden*; containing 2–4 fast-repeated double-notes in *N. hainanensis*; containing a significantly different first note in *N. daunchina* and *N. nankunensis*; containing a single note in *N. leishanensis*; (2) the call notes last 134.0–226.7 ms vs. the call notes last 30–54 ms in *N. yaoica*; (3) the calls of the southern lineage is similar to that of *N. adenopleura* s. s. but can be distinguished by the relative shorter note duration ( $164.3 \pm 16.2$  ms vs.  $212.3 \pm 33.0$  ms) and shorter note rise time ( $28.7 \pm 32.4$  ms vs.  $106.1 \pm 70.7$  ms).

The advertisement calls of the northern lineage is different from the congeners by (1) containing 2–7 ( $4.6 \pm 1.2$ ,  $N = 108$ ) identical regular notes vs. containing 10–25 fast-repeated regular notes in *Nidirana okinavana*; containing 2–4 fast-repeated double-notes in *N. hainanensis*; containing a significantly different first note in *N. daunchina* and *N. nankunensis*; containing a single note in *N. leishanensis*; (2) the call notes last 89.0–203.0 ms vs. the call notes last 30–54 ms in *N. yaoica*; (3) the calls of the southern lineage is similar to that of *N. adenopleura* s. s. but can be distinguished by the relative shorter note duration ( $136.9 \pm 23.2$  ms vs.  $212.3 \pm 33.0$  ms) and shorter



**Figure 3.** Advertisement call spectrograms. **(A)** *Nidirana adenopleura*; **(B)** *N. guangdongensis* sp. nov.; **(C)** *N. mangveni* sp. nov.; **(D)** *N. xiangica* sp. nov.; **(1)** sonogram; **(2)** waveform.

note rise time ( $79.5 \pm 26.9$  ms vs.  $106.1 \pm 70.7$  ms); (4) the calls of the southern lineage is similar to that of the southern lineage but can be distinguished by more note number in per call (2–7,  $4.6 \pm 1.2$  vs. 2–4,  $2.9 \pm 0.7$ ).

The advertisement calls of the western lineage is different from the congeners by (1) containing a significantly different first note vs. containing several identical regular notes in *Nidirana adenopleura*, southern lineage, northern lineage, *N. yaoica*, *N. chapaensis*, *N. lini*, and *N. pleuraden*; containing 2–4 fast-repeated double-notes in *N. hainanensis*; containing a single note in *N. leishanensis*; (2) containing 2–3 notes vs. containing 10–25 fast-repeated regular notes in *N. okinavana*; containing 13–15 fast-repeated notes in *N. nankunensis*; (3) the calls of the western lineage is similar to that of *N. daunchina* but can be distinguished by the relative shorter note intervals time ( $125.8 \pm 17.8$  ms vs.  $193.6 \pm 26.3$  ms) and shorter duration of non-first notes ( $74.6 \pm 11.8$  ms vs.  $140.6 \pm 5.6$  ms).

**Table 3.** Vocalization parameters of *Nidirana adenopleura*, *N. guangdongensis* sp. nov., *N. mangveni* sp. nov., and *N. xiangica* sp. nov.

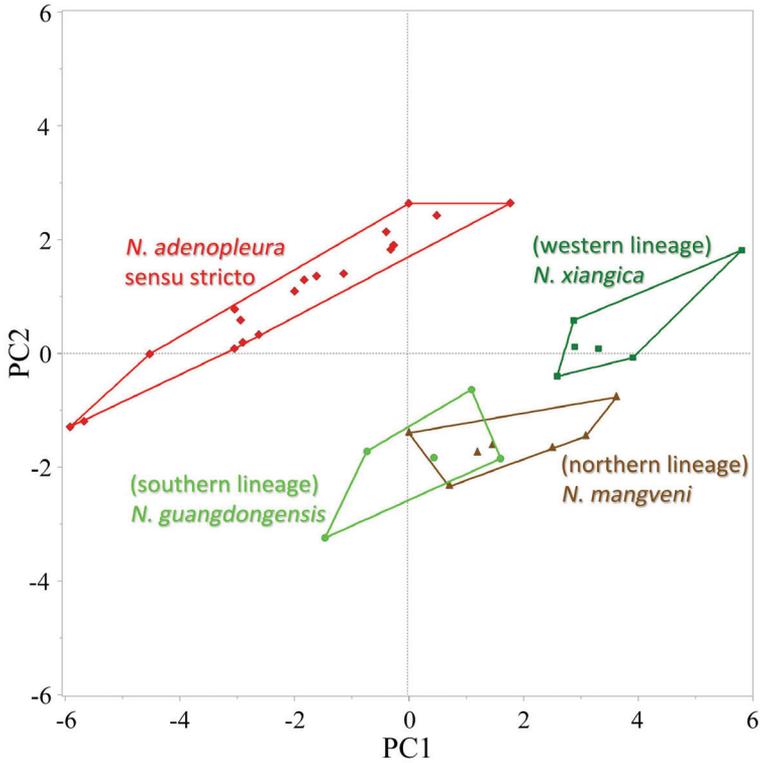
		<i>N. adenopleura</i>	<i>N. guangdongensis</i>	<i>N. mangveni</i>	<i>N. xiangica</i>
Call	Notes number	2–5 (3.4 ± 0.9, N = 83)	2–4 (2.9 ± 0.7, N = 54)	2–7 (4.6 ± 1.2, N = 108)	2–3 (2.8 ± 0.4, N = 57)
	Call duration (ms)	525.0–1585.5 (1005.1 ± 341.3, N = 83)	445.0–1198.1 (744.6 ± 206.8, N = 54)	423.6–1722.7 (967.2 ± 278.9, N = 108)	331.9–624.8 (504.3 ± 95.0, N = 57)
	Note duration (ms)	153.6–292.4 (212.3 ± 33.0, N = 260)	134.0–226.7 (164.3 ± 16.2, N = 150)	89.0–203.0 (136.9 ± 23.2, N = 462)	/
	Note rise time (ms)	1.4–228.3 (106.1 ± 70.7, N = 260)	0.0–138.5 (28.7 ± 32.4, N = 150)	4.1–148.6 (79.5 ± 26.9, N = 462)	/
	Note interval (ms)	104.0–245.2 (159.5 ± 28.4, N = 177)	79.9–262.6 (162.1 ± 26.4, N = 96)	59.3–192.7 (116.4 ± 20.8, N = 354)	85.0–195.6 (125.8 ± 17.8, N = 95)
First note	Note duration (ms)	/	/	/	148.0–233.0 (170.4 ± 14.5, N = 57)
	Note rise time (ms)	/	/	/	89.8–149.1 (126.2 ± 17.5, N = 57)
Non-first notes	Note duration (ms)	/	/	/	60.1–128.0 (74.6 ± 11.8, N = 95)
	Note rise time (ms)	/	/	/	2.2–43.0 (27.8 ± 10.2, N = 95)

## Morphology

The results of PCA based on morphometric measurements of the male specimens of *Nidirana adenopleura* s. s. and the three unnamed lineages are shown in Fig. 4. The extracted components PC1 eigenvectors accounted for 58.8% of the variance, PC2 for 18.6%, PC3 for 6.12%, and PC4 for 5.46%, which cumulate 88.98% of the variance. As shown on the scatter plot of PC1 and PC2, the specimens of *N. adenopleura* s. s., are significantly different from the specimens of the other three lineages. The specimens of the western lineage are also well separated from others. However, the specimens of the southern and northern lineages, which are significantly distant from each other in the phylogenetic tree, overlap with each other in the PCA result.

The results of one-way ANOVA and Tukey test for multiple comparisons are given in Table 4. The results indicate that all morphometric data are significantly different among *Nidirana adenopleura* s. s. and the three unnamed lineages (all  $p$ -values < 0.05). Specifically, for the specimens of *N. adenopleura* s. s. and the unnamed northern lineage which phylogenetically clustered within clade D together, they are significantly different in SVL, HDL, ED, RAD, FTL, and TIB. For the specimens of the unnamed western and southern lineages which phylogenetically clustered within clade C together, they are significantly different in HDW, SNT, IND, ED, TD, and RAD.

Detail comparisons among specimens of the western, southern, and northern lineages and all recognized congeners are listed in Table 5. The populations of the southern, northern, and western lineages can be readily and consistently distinguished from all other species by a combination of characteristics (see Comparisons below).



**Figure 4.** Scatter plot of PC1 and PC2 of Principal Component Analysis based on the morphometric measurements of male specimens of *Nidirana adenopleura*, *N. guangdongensis* sp. nov., *N. mangveni* sp. nov., and *N. xiangica* sp. nov.

**Table 4.** Morphometric comparisons based on the morphometric measurements of male specimens of *Nidirana adenopleura* ( $N = 18$ ), *N. guangdongensis* sp. nov. ( $N = 5$ ), *N. mangveni* sp. nov. ( $N = 7$ ), and *N. xiangica* sp. nov. ( $N = 6$ ). \* $p$ -values < 0.05, \*\* $p$ -values < 0.01, \*\*\* $p$ -values < 0.001.

	ANOVA	$p$ -value					
		<i>adenopleura</i> vs <i>guangdongensis</i>	<i>adenopleura</i> vs <i>mangveni</i>	<i>adenopleura</i> vs <i>xiangica</i>	<i>guangdongensis</i> vs <i>mangveni</i>	<i>guangdongensis</i> vs <i>xiangica</i>	<i>mangveni</i> vs <i>xiangica</i>
SVL	0.001 **	0.455	0.014 *	0.001 **	0.646	0.201	0.770
HDL	0.000 ***	0.886	0.004 **	0.002 **	0.133	0.074	0.980
HDW	0.000 ***	0.980	0.053	0.000 ***	0.336	0.005 *	0.164
SNT	0.009 *	1.000	0.993	0.006 *	0.997	0.044 *	0.041 *
IND	0.001 **	1.000	0.237	0.001 **	0.436	0.007 *	0.141
IOD	0.013 *	0.975	0.501	0.008 *	0.890	0.109	0.292
ED	0.000 ***	0.068	0.000 ***	0.000 ***	0.028 *	0.012 *	0.963
TD	0.001 **	0.999	0.052	0.001 **	0.159	0.011 *	0.533
TED	0.005 *	0.784	0.065	0.203	0.656	0.107	0.003 **
HND	0.017 *	0.582	0.308	0.013 *	0.995	0.458	0.530
RAD	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.729	0.027 *	0.158
FTL	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.976	0.278	0.092
TIB	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.997	0.371	0.413

**Table 5.** Diagnostic characters separating all species of genus *Nidirana*.

Species	SVL of males (mm)	SVL of females (mm)	Fingers tips	Lateroventral groove on fingers	Relative length of fingers	Toes tips	Lateroventral groove on toes	Tibio-tarsal articulation	Subgular vocal sacs	Nuptial pad
<i>N. guangdongensis</i>	50.0–58.4	55.3–59.3	Dilated	Present except finger I	II < I < IV < III	Dilated	Present	Nostril	Present	One on finger I
<i>N. mangvoni</i>	53.6–59.7	59.7–65.1	Dilated	Present on fingers III and IV	I < II < IV < III	Dilated	Present	Anterior corner of eye	Present	One on finger I
<i>N. xiangica</i>	56.3–62.3	53.5–62.6	Dilated	Present	II < I < IV < III	Dilated	Present	Eye-snout	Present	One on finger I
<i>N. adenopleura</i>	43.1–57.6	47.6–60.7	Dilated	Present except finger I	II < I < IV < III	Dilated	Present	Snout tip or eye-snout	Present	One on finger I
<i>N. nankunensis</i>	33.3–37.1	37.8–39.5	Dilated	Present except finger I	II < I < IV < III	Dilated	Present	Nostril	Present	One on finger I
<i>N. okinavana</i>	35.5–42.8	44.6–48.8	Dilated	Present except finger I	II < I < IV < III	Dilated	Present	Eye center-near nostril	Absent	Poorly one on finger I
<i>N. daunchina</i>	40.6–51.0	44.0–53.0	Dilated	Absent or rarely present	II < I < IV < III	Dilated	Present	Nostril	Present	One on finger I
<i>N. yaocia</i>	40.4–45.9	?	Dilated	Present	II < I < IV < III	Dilated	Present	Nostril	Present	One on finger I
<i>N. chapensis</i>	32.8–42.5	41.0–51.8	Dilated	Present except finger I	II < I < IV < III	Dilated	Present	Nostril	Present	Two on finger I
<i>N. hainanensis</i>	32.8–44.4	?	Dilated	Present	II < I < IV < III	Dilated	Present	Nostril	Present	Absent
<i>N. leishanensis</i>	49.5–56.4	43.7–55.3	Dilated	Present	II < IV < I < III	Dilated	Present	Eye-snout	Present	Two on fingers I and II
<i>N. lini</i>	44.1–63.1	57.7–68.6	Dilated	Present except finger I	II < I < IV < III	Dilated	Present	Beyond snout	Present	One on finger I
<i>N. pleuraden</i>	45.4–58.7	45.5–62.5	Not dilated	Absent	II < I < IV < III	Not dilated	Absent	Eye-snout	Present	One on finger I

**Table 5.** Continued.

Species	Spicules on dorsal skin	Nest construction	Tadpole labial tooth row formula	Calling	Cites
<i>N. guangdongensis</i>	Entire	Absent	?	2–4 regular notes	This study
<i>N. mangvoni</i>	Entire or posterior	Absent	?	2–7 regular notes	This study
<i>N. xiangica</i>	Entire	Absent	?	2–3 notes containing a specific first note	This study
<i>N. adenopleura</i>	Entire or posterior	Absent	1:1+1/1+1.2 or 1:0+0/1+1:1	2–5 regular notes	Pope (1931); Chuaynkern et al. (2010); Lyu et al. (2017); this study
<i>N. nankunensis</i>	Absent or few above vent	Present	1:1+1/1+1.2	13–15 fast-repeated notes containing a specific first note	Lyu et al. (2017)
<i>N. okinavana</i>	Absent	Present	1:1+1/1+1.2	10–25 fast-repeated notes	Matsui and Ursumiya (1983); Chuaynkern et al. (2010)
<i>N. daunchina</i>	Absent	Present	1:1+1/1+1.2 or 1:1+1/2+2:1	2–5 notes containing a specific first note	Liu (1950); Fei et al. (2009); Lyu et al. (2017)
<i>N. yaocia</i>	Absent	?	?	1–3 fast-repeated regular notes	Lyu et al. (2019)
<i>N. chapensis</i>	Absent or few above vent	Present	1:1+2/1+1.2	3 notes	Chuaynkern et al. (2010)
<i>N. hainanensis</i>	Absent	Present	?	2–4 fast-repeated double-notes	Fei et al. (2009, 2012); Lyu et al. (2017)
<i>N. leishanensis</i>	Absent	Absent	1:1+2/1+1.2	1 single note	Li et al. (2019)
<i>N. lini</i>	Posterior	Absent	1:1+1/1+1.2	5–7 notes	Chou (1999); Fei et al. (2009); Lyu et al. (2017)
<i>N. pleuraden</i>	Posterior	Absent	1:1+1/1+1.2 or 1:1+1/2+2:1	4–7 notes	Fei et al. (2009); Lyu et al. (2017)

## Conclusion

Based on the results of molecular, bioacoustic, and morphological analyses, the populations of the southern, northern and western lineages are significantly different from all congeners of genus *Nidirana*, including the *N. adenopleura* s. s. Thus, we propose these three lineages as three new species, i.e., *Nidirana guangdongensis* sp. nov. for the population from Nanling Mountains and southern Luoxiao Mountains (southern lineage), *Nidirana mangveni* sp. nov. for the population from northern Zhejiang (northern lineage), and *Nidirana xiangica* sp. nov. for the population from Xiangjiang River Basin (western lineage).

## Taxonomic accounts

### *Nidirana guangdongensis* Lyu, Wan, & YY Wang, sp. nov.

<http://zoobank.org/52CE0A4A-BDC1-4E5B-B2C3-7A58FDABE24F>

Guangdong Music Frog / Yuè Qín Wā (粤琴蛙)

Figures 5–7

**Chresonymy.** *Nidirana adenopleura*: Fei et al. 2009, 2012; Li et al. 2011

**Holotype.** SYS a005767 (Figs 5, 6), adult male, collected by Zhi-Tong Lyu on 24 April 2017 from Shimentai Nature Reserve (24.4450°N, 113.1617°E; ca. 320 m a.s.l.), Yingde City, Guangdong Province, China.

**Paratypes.** Seven adult specimens from the same locality as the holotype. Male SYS a005765 and female SYS a005766, collected by Zhi-Tong Lyu and Yuan-Qiu Li at the same time as the holotype; male SYS a005995 and females SYS a005997–98, collected by Zhi-Tong Lyu, Yong-You Zhao and Chao-Yu Lin on 20 June 2017; male SYS a006879/CIB 107273 collected by Zhi-Tong Lyu, Yong-You Zhao and Yuan-Qiu Li on 20 April 2018; male SYS a007688 collected by Yu-Long Li, Can-Zhong Rong and Yuan-Qiu Li on 23 April 2019.

**Etymology.** The species name *guangdongensis* refers to Guangdong (广东), also known as Yue (粤), which is the province where the type locality, Shimentai Nature Reserve, belongs to.

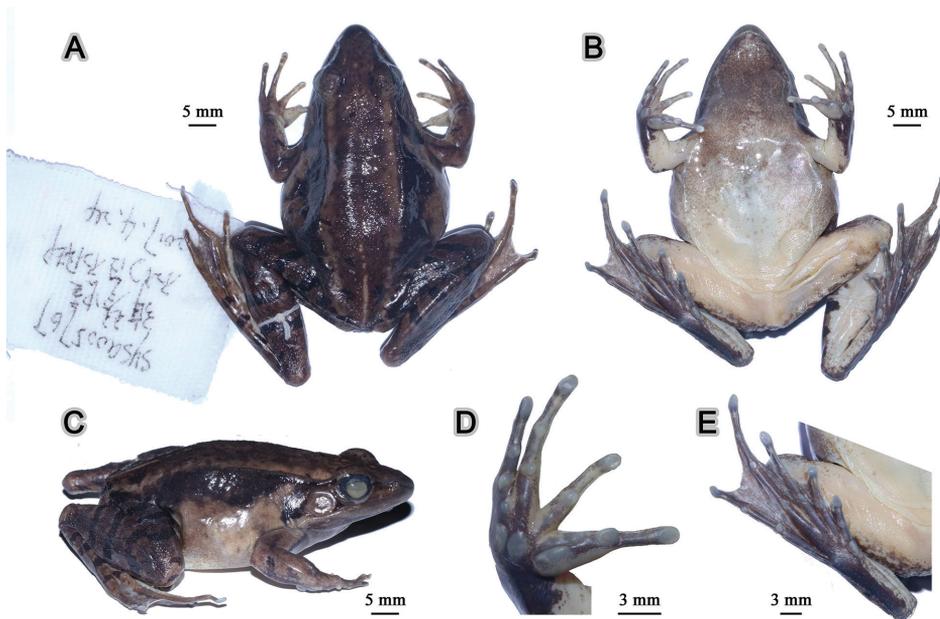
**Differential diagnosis.** *Nidirana guangdongensis* sp. nov. is distinguished from its congeners by the following combination of the morphological characteristics: (1) body large and elongated, with SVL 50.0–58.4 ( $53.9 \pm 3.3$ ,  $N = 5$ ) mm in adult males, and SVL 55.3–59.3 ( $57.0 \pm 2.1$ ,  $N = 3$ ) mm in adult females; (2) disks of digits dilated, rounded; (3) lateroventral grooves present on every digit except finger I; (4) heels overlapping; (5) tibio-tarsal articulation reaching the nostril; (6) mid-dorsal stripe present on posterior dorsum; (7) weak supernumerary tubercles below the base of each finger, palmar tubercles prominent and distinct; (8) supratympanic fold absent; (9) white horny spinules on the entirely dorsum, dorsolateral folds, flanks and dorsal hindlimbs, while absent on temporal regions in males; (10) a pair of subgular



**Figure 5.** Morphological features of the adult male holotype SYS a005767 of *Nidirana guangdongensis* sp. nov. in life. (A) dorsolateral view; (B) ventral view; (C) left hand; (D) nuptial pad; (E) right foot; (F) close-up of head showing the dense white horny spinules on dorsum, upper eyelid, while absent on temporal regions.

vocal sacs present; (11) one single nuptial pad present on the finger I, nuptial spinules invisible; (12) suprabrachial gland large and smooth, prominent; (13) calling: 2–4 identical regular notes.

**Comparison.** Morphologically, *Nidirana guangdongensis* sp. nov. is unique when compared with all known congeners by the combination of the following characteristics: (1) large body size, SVL 50.0–58.4 mm in males and 55.3–59.3 mm in females vs. < 48.0 mm in males or < 53.0 mm in females in *N. nankunensis*, *N. okinavana*, *N.*



**Figure 6.** Morphological features of the adult male holotype SYS a005767 of *Nidirana guangdongensis* sp. nov. in preservative. (A) dorsal view; (B) ventral view; (C) lateral view; (D) right hand; (E) right foot.

*daunchina*, *N. yaoica*, *N. chapaensis* and *N. hainanensis*; (2) relative finger lengths  $II < I < IV < III$  vs.  $II < I = IV < III$  in *N. chapaensis*; vs.  $II < IV < I < III$  in *N. leishanensis*; (3) presence of lateroventral groove on every digit except finger I vs. absent on fingers and toes in *N. pleuraden*; vs. absent or barely visible on fingers in *N. daunchina*; vs. present on finger I in *N. yaoica*, *N. leishanensis* and *N. hainanensis*; (4) tibio-tarsal articulation reaches at the nostril vs. beyond the snout tip in *N. lini*; (5) white horny spinules on the entirely dorsum and flanks in males vs. absent on dorsum and flanks or few above vent in *N. nankunensis*, *N. okinavana*, *N. daunchina*, *N. yaoica*, *N. chapaensis*, *N. leishanensis* and *N. hainanensis*; vs. present on dorsum while absent on flanks in *N. adenopleura*, *N. lini* and *N. pleuraden*; (6) the presence of a single nuptial pad on finger I vs. absent in *N. hainanensis*; vs. divided into two parts in *N. chapaensis*; vs. two nuptial pads on fingers I and II respectively; (7) the presence of a pair of subgular vocal sacs vs. absent in *N. okinavana*.

**Description of holotype.** SYS a005767 (Figs 5, 6), adult male. Body large and elongated, SVL 55.2 mm; head longer than wide (HDW/HDL 0.90), flat above; snout rounded in dorsal and lateral views, slightly protruding beyond lower jaw, longer than horizontal diameter of eye (SNT/ED 1.30); canthus rostralis distinct, loreal region concave; nostril round, directed laterally, closer to the snout than to the eye; a longitudinal swollen mandibular ridge extending from below nostril through lower edges of eye and tympanum to above insertion of arm, where the ridge is intermittent, forming a maxillary gland and shoulder gland; supratympanic fold absent; interorbital space flat, narrower than internasal distance (IND/IOD



**Figure 7.** (A) adult female paratype SYS a005766 of *Nidirana guangdongensis* sp. nov.; (B) adult male paratype SYS a005765; (C) eggs in the water surface found in Mt Bamian.

1.24); pupil elliptical, horizontal; tympanum distinct, round, TD/ED 0.86, and close to eye, TED/TD 0.32; pineal ocellus slightly visible; vomerine ridge present, bearing small teeth; tongue large, cordiform, notched behind; a pair of subgular vocal sacs present.

Forelimbs moderately robust, lower arm 0.17 of SVL and hand 0.27 of SVL; fingers thin, relative finger lengths II < I < IV < III; tip of each finger slightly dilated, forming rounded disks; lateroventral grooves on all fingers except finger I, not meeting at the tip of disks; fingers free of webbing; presence of distinct lateral fringes on inner and outer sides of fingers II, III and IV, and on outer side of finger I; subarticular tubercles prominent and rounded; weak supernumerary tubercles below the base of each finger; three elliptic, large, prominent and very distinct palmar tubercles; a single nuptial pad on the dorsal surface of first finger, nuptial spinules invisible.

Hindlimbs relatively robust, tibia 0.54 of SVL and foot 0.77 of SVL; heels overlapping when hindlimbs flexed at right angles to axis of body; tibio-tarsal articulation reaching the nostril when hindlimb is stretched along the side of the body; toes

relatively long and thin, relative lengths  $I < II < V < III < IV$ ; tip of each toe slightly dilated with remarkable elongated ventral callous pad, forming long and pointed disk; well-developed lateroventral grooves on toes, not meeting at the tip of disks; webbing moderate, webbing formula:  $I 1\frac{1}{3} - 2 II 1\frac{1}{3} - 2\frac{1}{3} III 1\frac{2}{3} - 3 IV 3\frac{1}{3} - 1\frac{1}{3} V$ ; presence of lateral fringes on inner and outer sides of each toes, forming distinct dermal flap on the lateral edges of toes I and V; subarticular tubercles rounded, prominent; inner metatarsal tubercle elliptic, length triple the width; outer metatarsal tubercle indistinct, small and rounded; tarsal folds and tarsal tubercle absent.

Dorsal surface rough with dense horny spinules; developed dorsolateral fold with sparse horny spinules from posterior margin of upper eyelid to above groin but intermittent posteriorly; flank rough with dense tubercles and dense horny spinules; a large and smooth suprabrachial gland behind base of forelimb, prominent; dorsal surface of forelimb relatively smooth without horny spinules, weak longitudinal ridges on upper arms and slightly extending to lower arm; the dorsal surfaces of thigh and tibia rough with dense tubercles and dense horny spinules, forming several longitudinal ridges. Ventral surface of throat, body, and limbs smooth; large flattened tubercles densely arranged on the rear of thigh and around vent.

**Coloration of holotype.** In life (Fig. 5), dorsal surface reddish brown; horny spinules on the skin white; pineal ocellus yellowish; a yellowish mid-dorsal stripe on the posterior dorsum; dorsolateral fold dark brown; upper flank dark brown; lower flank light brown; suprabrachial gland light brown. Dorsal forelimbs light brown; a longitudinal black stripe on the anterior surface of the forelimb; dorsal hindlimbs dark brown, four dark crossbars on the thigh, three on the tibia and three on the tarsus. Loreal and temporal regions dark brown, tympanum light brown; upper  $\frac{1}{3}$  iris brownish white and lower  $\frac{2}{3}$  iris reddish brown; maxillary gland and shoulder gland white. Throat dark purplish brown; ventral surface of body and limbs creamy white; rear thigh tinged with pink; ventral hand white with large purplish brown patches; ventral foot purplish brown.

In preservative (Fig. 6), dorsal surface faded with the pineal ocellus and mid-dorsal stripe clearer; white spinules more distinct; dorsal limbs faded with the crossbars more distinct; ventral surface faded, throat grey.

**Variations.** Measurements of type series are given in Table 6. All specimens were similar in morphology. Females ( $57.0 \pm 2.1$  mm,  $N = 3$ ) (Fig. 7A) are not significantly larger than males ( $53.9 \pm 3.3$  mm,  $N = 5$ ), but relatively smooth than males, not bearing white horny spinules on the dorsum, dorsolateral folds, and flanks. Pineal ocellus invisible in SYS a005765 (Fig. 7B); numerous black spots on flanks in SYS a005766.

**Distribution and ecology.** Currently, *Nidirana guangdongensis* sp. nov. is known from northern Guangdong, southern Jiangxi and southeastern Hunan, indicating that this frog is distributed in the Nanling Mountains and southern Luoxiao Mountains of southern China. The frog inhabits in natural ponds. The adult males call at the water surface and the females oviposit directly into the water (Fig. 7C) from April to June. The tadpoles of this species remain unknown. In Mt Nankun, *N. guangdongensis* sp. nov. is sympatric with *N. nankunensis* in the same pond and is more abundant.

**Table 6.** Measurements (in mm) of the type series of *Nidirana guangdongensis* sp. nov. An asterisk denotes the holotype.

	SYS a005767*	SYS a005765	SYS a005995	SYS a006879	SYS a007688	SYS a005766	SYS a005997	SYS a005998
Sex	Male	Male	Male	Male	Male	Female	Female	Female
SVL	55.2	51.3	50.0	58.4	54.6	56.4	59.3	55.3
HDL	19.4	18.9	18.5	20.8	20.3	20.6	22.2	21.0
HDW	17.5	17.4	17.7	18.5	18.0	18.1	18.6	18.5
SNT	7.9	7.8	7.4	7.9	8.0	8.1	8.6	8.0
IND	5.7	5.8	5.3	5.8	5.6	5.9	6.2	5.5
IOD	4.6	4.7	4.3	4.9	4.9	5.4	5.2	5.1
ED	6.1	5.3	5.6	5.9	5.4	6.1	6.1	5.8
TD	5.2	4.1	3.8	4.9	4.1	4.6	4.2	4.7
TED	1.7	1.5	1.3	1.2	1.3	1.5	1.2	1.4
HND	14.7	13.3	13.8	14.4	14.3	14.4	14.6	15.8
RAD	9.4	8.7	8.6	9.9	8.9	9.0	9.7	9.7
FTL	42.7	39.0	40.1	45.3	43.9	45.5	46.9	47.0
TIB	29.6	27.0	25.4	30.0	29.2	30.1	31.6	31.9

**Vocalization.** The advertisement call ( $N = 54$ ) of *Nidirana guangdongensis* sp. nov. contains 2–4 repeated, identical, regular notes. The two-note call has a duration of 445.0–559.0 ( $520.6 \pm 27.4$ ,  $N = 19$ ) ms; the three-note call has a duration of 681.5–875.8 ( $794.6 \pm 46.4$ ,  $N = 28$ ) ms; the four-note call has a duration of 1117.6–1198.1 ( $1152.9 \pm 29.8$ ,  $N = 7$ ) ms. The notes last 134.0–226.7 ( $164.3 \pm 16.2$ ,  $N = 150$ ) ms with the rise time 0.0–138.5 ( $28.7 \pm 32.4$ ,  $N = 150$ ) ms, and the intervals last 79.9–262.6 ( $162.1 \pm 26.4$ ,  $N = 96$ ) ms.

***Nidirana mangveni* Lyu, Qi, & YY Wang, sp. nov.**

<http://zoobank.org/D4BC572F-FAA8-41A8-856D-3D183FA2AC09>

Mangven Chang's Music Frog / Mèng Wén Qín Wā (孟闻琴蛙)

Figures 8–10

**Chresonymy.** *Nidirana adenopleura*: Fei et al. 2009, 2012

**Holotype.** SYS a006313 (Figs 8, 9), adult male, collected by Jian Wang and Zhao-Chi Zeng on 1 August 2017 from Mt Dapan (28.9801°N, 120.5447°E; ca 860 m a.s.l.), Pan'an County, Zhejiang Province, China.

**Paratypes.** Eight adult specimens. Males SYS a006311–12, SYS a006314/CIB 107275, and female SYS a006310, collected by Jian Wang and Zhao-Chi Zeng at the same time from the same locality as the holotype; males SYS a006413–14 and female SYS a006416, collected by Jian Wang and Zhao-Chi Zeng on 3 August 2017 from Mt Longmen (29.8643°N, 119.9790°E; ca 540m a.s.l.), Fuyang District, Hangzhou City, Zhejiang Province, China; male SYNU 12050569 collected by Zheng-Yan Zhou on 8 May 2012 from Hangzhou Botanical Garden (30.2544°N, 120.1226°E; ca 100m a.s.l.), Xihu District, Hangzhou City, Zhejiang Province, China.

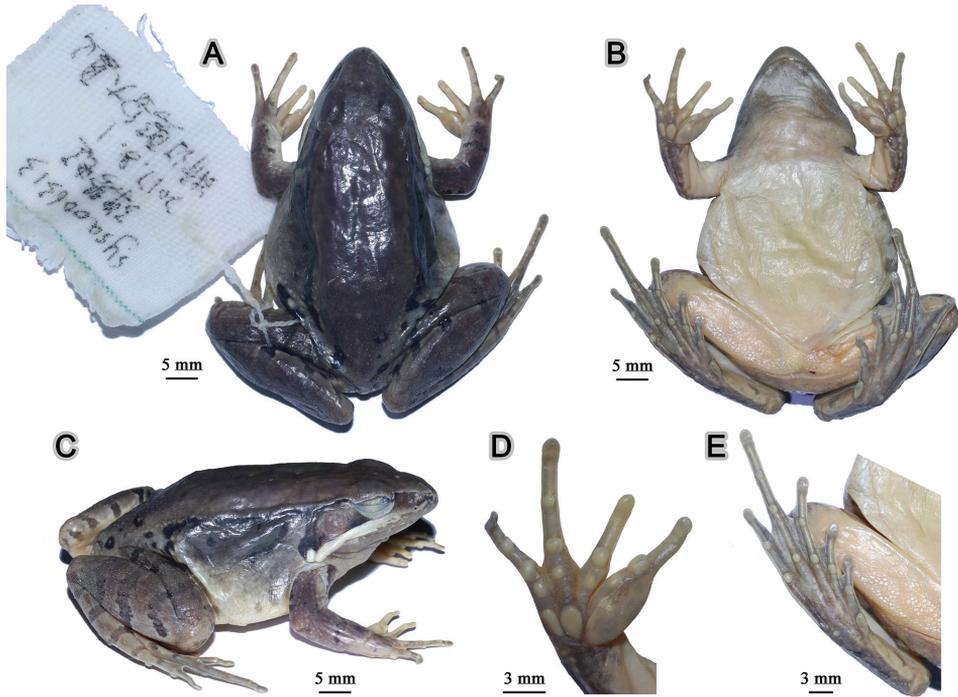
**Etymology.** The species name *mangveni* refers to Professor Mangven L. Y. Chang (= Meng-Wen Zhang, 张孟闻), an outstanding zoologist born in Ningbo City of north-



**Figure 8.** Morphological features of the adult male holotype SYS a006313 of *Nidirana mangveni* sp. nov. in life. (A) dorsolateral view; (B) ventral view; (C) right hand; (D) left foot; (E) close-up of head showing the weak supratympanic fold; (F) close-up of posterior dorsum and hindlimb showing the horny spinules.

ern Zhejiang, who contributed mostly on Chinese herpetological taxonomy and natural history. He is also the author of *Nidirana daunchina*, a congener of this new species.

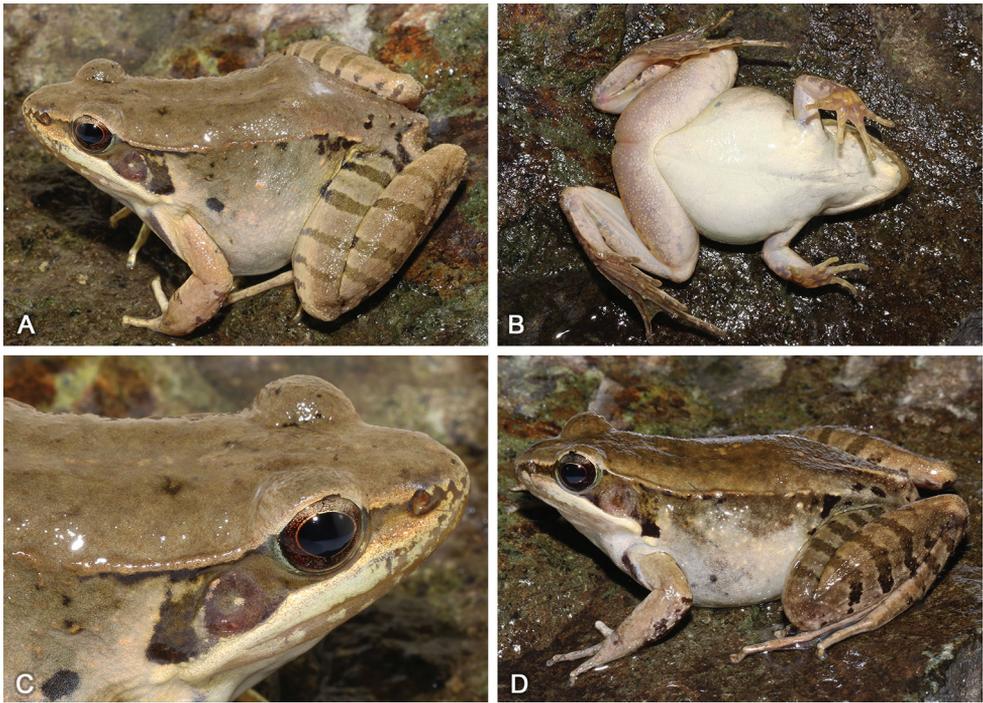
**Differential diagnosis.** *Nidirana mangveni* sp. nov. is distinguished from its congeners by the following combination of the morphological characteristics: (1) body large and elongated, with SVL 53.6–59.7 ( $56.2 \pm 2.5$ ,  $N = 7$ ) mm in adult males, and SVL 62.4  $\pm$  3.8 (59.7–65.1,  $N = 2$ ) mm in adult females; (2) disks of digits dilated, rounded; (3) lateroventral grooves present on fingers III and IV, and each toes; (4) relative finger lengths I < II < IV < III; (5) heels overlapping; (6) tibio-tarsal articulation reaching the anterior corner of eye; (7) weak supratympanic fold present; (8)



**Figure 9.** Morphological features of the adult male holotype SYS a006313 of *Nidirana mangveni* sp. nov. in preservative. (A) dorsal view; (B) ventral view; (C) lateral view; (D) right hand; (E) right foot.

mid-dorsal stripe absent or present on posterior dorsum; (9) posterior of dorsal skin rough with dense tubercles; (10) developed supernumerary tubercles below the base of each finger, palmar tubercles prominent and distinct; (11) white horny spinules on the posterior or entire dorsum in males; (12) a pair of subgular vocal sacs present; (13) one single nuptial pad present on the finger I, nuptial spinules invisible; (14) suprabrachial gland large; (15) calling: 2–7 identical regular notes.

**Comparison.** Morphologically, *Nidirana mangveni* sp. nov. is unique when compared with all recognized congeners by the combination of the following characteristics: (1) large body size, SVL 53.6–59.7 mm in males and 59.7–65.1 mm in females vs. < 53.0 mm in males or females in *N. nankunensis*, *N. okinavana*, *N. daunchina*, *N. yaoica*, *N. chapaensis* and *N. hainanensis*; (2) relative finger lengths  $I < II < IV < III$  vs.  $II < I = IV < III$  in *N. chapaensis*; vs.  $II < IV < I < III$  in *N. leishanensis*; vs.  $II < I < IV < III$  in all other congeners; (3) absent of lateroventral groove on fingers I and II vs. absent on fingers and toes in *N. pleuraden*; vs. absent or barely visible on fingers in *N. daunchina*; vs. present on finger II in all other congeners; (4) tibio-tarsal articulation reaches at the anterior corner of eye vs. beyond the snout tip in *N. lini*; vs. at the nostril in *N. guangdongensis*, *N. nankunensis*, *N. daunchina*, *N. yaoica*, *N. chapaensis* and *N. hainanensis*; (5) weak supratympanic fold present vs. absent in *N. guangdongensis*, *N. adenopleura*, *N. nankunensis*, *N. daunchina*, *N. yaoica*, *N. hainanensis*, and *N. lini*; (6)



**Figure 10.** (A), (B), and (C) dorsolateral view, ventral view, close-up of head showing the week supratympanic fold of adult female paratype SYS a006310 of *Nidirana mangveni* sp. nov.; (D) adult female paratype SYS a006416.

white horny spinules on the posterior or entire dorsum in males vs. absent on dorsum or few above vent in *N. nankunensis*, *N. okinavana*, *N. daunchina*, *N. yaoica*, *N. chapaensis*, *N. leishanensis* and *N. hainanensis*; (7) the presence of a single nuptial pad on finger I vs. absent in *N. hainanensis*; vs. divided into two parts in *N. chapaensis*; vs. two nuptial pads on fingers I and II respectively; (8) the presence of a pair of subgular vocal sacs vs. absent in *N. okinavana*.

**Description of holotype.** SYS a006313 (Figs 8, 9), adult male. Body large and elongated, SVL 54.0 mm; head longer than wide (HDW/HDL 0.87), flat above; snout rounded in dorsal and lateral views, slightly protruding beyond lower jaw, longer than horizontal diameter of eye (SNT/ED 1.22); canthus rostralis distinct, loreal region concave; nostril round, directed laterally, closer to the snout than to the eye; a longitudinal swollen mandibular ridge extending from below nostril through lower edges of eye and tympanum to above insertion of arm, where the ridge is intermittent, forming a maxillary gland and shoulder gland; week supratympanic fold present; interorbital space flat, narrower than internasal distance (IND/IOD 1.25); pupil elliptical, horizontal; tympanum distinct, round, TD/ED 0.73, and close to eye, TED/TD 0.31; pineal ocellus present; vomerine ridge present, bearing small teeth; tongue large, cordiform, notched behind; a pair of subgular vocal sacs present.

Forelimbs moderately robust, lower arm 0.18 of SVL and hand 0.26 of SVL; fingers thin, relative finger lengths  $I < II < IV < III$ ; tip of each finger slightly dilated, forming rounded disks; lateroventral grooves on fingers III and IV, not meeting at the tip of disks; fingers free of webbing; presence of distinct lateral fringes on inner and outer sides of fingers II, III and IV, absent on finger I; subarticular tubercles prominent and rounded; developed supernumerary tubercles below the base of each finger; three elliptic, large, prominent and very distinct palmar tubercles; a single nuptial pad on the dorsal surface of first finger, nuptial spinules invisible.

Hindlimbs relatively robust, tibia 0.52 of SVL and foot 0.76 of SVL; heels overlapping when hindlimbs flexed at right angles to axis of body; tibio-tarsal articulation reaching the anterior corner of eye when hindlimb is stretched along the side of the body; toes relatively long and thin, relative lengths  $I < II < V < III < IV$ ; tip of each toe slightly dilated with remarkable elongated ventral callous pad, forming long and pointed disk; well-developed lateroventral grooves on toes, not meeting at the tip of disks; webbing moderate, webbing formula:  $I \frac{1}{2} - \frac{2}{3} \quad II \frac{1}{3} - \frac{2}{3} \quad III \frac{1}{2} - 3 \quad IV \frac{3}{3} - 1\frac{2}{3} \quad V$ ; presence of lateral fringes on inner and outer sides of each toes, forming distinct dermal flap on the lateral edges of toes I and V; subarticular tubercles rounded, prominent; inner metatarsal tubercle elliptic, length triple the width; outer metatarsal tubercle indistinct, small and rounded; tarsal folds and tarsal tubercle absent.

Dorsal skin of head and anterior body smooth, posterior dorsum of body rough with dense tubercles with horny spinules; weak intermittent dorsolateral fold from posterior margin of upper eyelid to above groin; upper flank with sparse tubercles; a large and smooth suprabrachial gland behind base of forelimb, not prominent; dorsal surface of upper arm smooth with sparse tubercles without spinules; the dorsal surfaces of thigh and tibia relatively rough with several weak longitudinal ridges and tubercles bearing spinules. Ventral surface of throat, body, and limbs smooth; large flattened tubercles densely arranged on the rear of thigh and around vent.

**Coloration of holotype.** In life (Fig. 8), dorsal surface brown; pineal ocellus yellowish; mid-dorsal stripe unclear; dorsolateral fold dark brown; upper flank olive brown; lower flank creamy white; suprabrachial gland white. Dorsal limbs brown; a longitudinal black stripe on the anterior surface of the forelimb; three dark crossbars on the thigh, three on the tibia and three on the tarsus. Loreal and temporal regions dark, tympanum light brown; upper  $\frac{1}{3}$  iris brownish white and lower  $\frac{2}{3}$  iris reddish brown; maxillary gland and shoulder gland white. Throat white tinged with pink, but two subgular vocal sacs flesh colored; ventral surface of body and limbs creamy white; rear thigh tinged with pink; ventral hand flesh colored; ventral foot brown.

In preservative (Fig. 9), dorsal surface became darker; mid-dorsal stripe unclear; white spinules more distinct; pineal ocellus more distinct; crossbars on limbs became clearer; flanks and ventral surface faded.

**Variations.** Measurements of type series are given in Table 7. All specimens were similar in morphology. Females ( $62.4 \pm 3.8$  mm,  $N = 2$ ) (Fig. 10) are relatively larger than males ( $56.2 \pm 2.5$  mm,  $N = 7$ ), and more smooth than males. Pineal ocellus invisible in SYS a006310, 6311; dorsal surface light brown in SYS a006310, 6311; a short

**Table 7.** Measurements (in mm) of the type series of *Nidirana mangveni* sp. nov. An asterisk denotes the holotype.

	SYS a006313 *	SYS a006311	SYS a006312	SYS a006314 /CIB 107275	SYS a006413	SYS a006414	SYNU 12050569	SYS a006310	SYS a006416
Sex	Male	Male	Male	Male	Male	Male	Male	Female	Female
SVL	54.0	58.2	56.2	53.7	59.7	57.9	53.6	59.7	65.1
HDL	20.8	21.2	20.2	20.0	22.3	21.6	21.9	21.9	24.6
HDW	18.0	19.4	18.1	18.1	20.2	20.0	18.4	19.4	19.1
SNT	7.7	7.8	7.5	7.7	7.9	8.4	8.0	8.7	8.6
IND	6.0	6.6	6.0	6.1	6.6	6.0	5.1	6.6	6.7
IOD	4.8	5.2	5.1	4.4	5.4	4.5	4.4	5.2	5.2
ED	6.3	6.5	6.4	6.4	6.1	6.3	5.6	6.4	7.0
TD	4.6	5.5	4.6	4.8	5.6	5.5	4.7	5.3	5.7
TED	1.4	1.2	1.4	1.3	1.3	1.2	1.5	1.4	1.6
HND	14.3	15.0	14.5	13.8	14.7	14.4	12.8	15.1	14.7
RAD	9.5	10.0	9.8	9.6	10.1	10.0	10.0	10.8	11.0
FTL	41.0	43.8	41.7	40.0	45.4	40.9	39.2	44.2	47.7
TIB	27.8	30.0	28.5	27.2	30.0	27.8	28.2	29.4	33.7

mid-dorsal stripe on the posterior dorsum in SYS a006311, 6416; spinules on the entire dorsum in SYS a006413.

**Distribution and ecology.** Currently, *Nidirana mangveni* sp. nov. is known from Mt Dapan, Mt Longmen, and Hangzhou Botanical Garden, all situated in northern Zhejiang, suggesting the *Nidirana* populations in northern Zhejiang might belong to this species. This frog inhabits natural or artificial swamps, ponds, and paddy fields. The adult males do not construct nests and calls at the water surface or the bank from May to August. The male individual SYNU12050569 which was found in early May bears indistinct nuptial pads but processes the suprabrachial gland, indicating the breeding season of this species begins from early May. The tadpoles of this species remain unknown.

**Vocalization.** The advertisement call ( $N = 108$ ) of *Nidirana mangveni* sp. nov. contains 2–7 repeated, identical, regular notes. The three-note call has a duration of 515.0–741.0 ( $684.0 \pm 50.9$ ,  $N = 26$ ) ms; the four-note call has a duration of 722.5–1044.6 ( $907.0 \pm 82.9$ ,  $N = 40$ ) ms; the five-note call has a duration of 898.1–1341.7 ( $1087.1 \pm 108.5$ ,  $N = 20$ ) ms; the six-note call has a duration of 1332.0–1427.0 ( $1377.9 \pm 26.4$ ,  $N = 15$ ) ms. The notes last 89.0–203.0 ( $136.9 \pm 23.2$ ,  $N = 462$ ) ms with the rise time 4.1–148.6 ( $79.5 \pm 26.9$ ,  $N = 462$ ) ms, and the intervals last 59.3–192.7 ( $116.4 \pm 20.8$ ,  $N = 354$ ) ms.

***Nidirana xiangica* Lyu & YY Wang, sp. nov.**

<http://zoobank.org/855B3537-8FFE-408D-8062-B0D9EF3C680A>

Xiangjiang Music Frog / Xiāng Qín Wā (湘琴蛙).

Figures 11–13

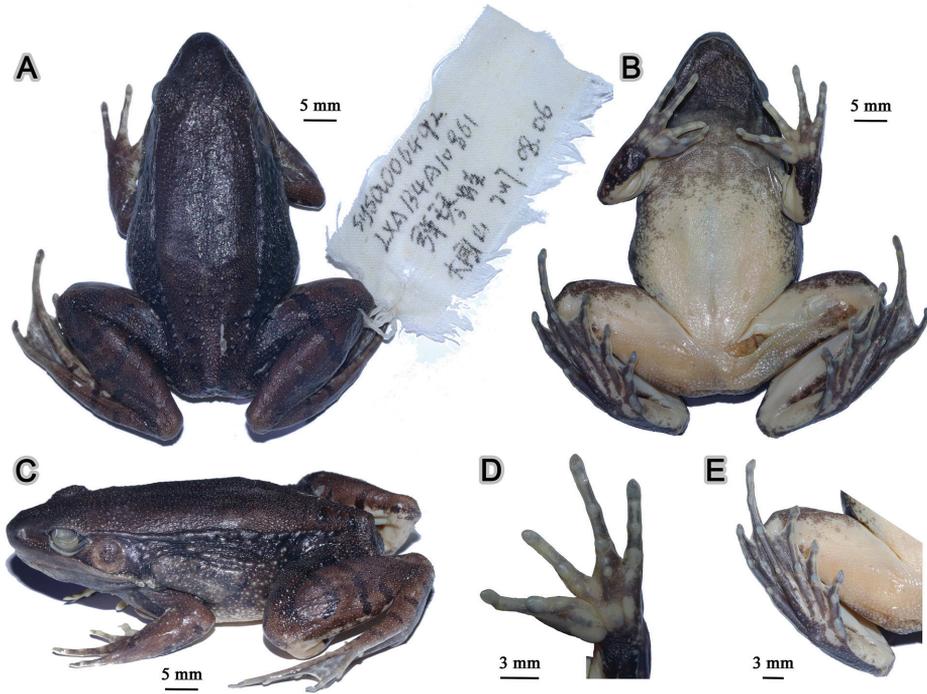
**Chresonymy.** *Nidirana adenopleura*: Fei et al. 2009, 2012; Mo et al. 2014.



**Figure 11.** Morphological features of the adult male holotype SYS a006492 of *Nidirana xiangica* sp. nov. in life. (A) dorsolateral view; (B) ventral view; (C) right hand; (D) nuptial pad; (E) left foot; (F) close-up of head showing the dense white horny spinules on dorsum, upper eyelid, loreal region, and temporal region including tympanum.

**Holotype.** SYS a006492 (Figs 11, 12), adult male, collected by Zhi-Tong Lyu on 6 August 2018 from Mt Dawei (28.4237°N, 114.0793°E; ca 820 m a.s.l.), Liuyang City, Hunan Province, China.

**Paratypes.** Nine adult specimens. Male SYS a006493/CIB 107276 and female SYS a006491, collected by Zhi-Tong Lyu and Zheng-Jiao Liu at the same time from the same locality as the holotype; male SYS a002591 and female SYS a002590, collected by Jian Zhao on 8 May 2014 from Mt Wugong (27.4079°N, 114.1671°E; ca 800 m a.s.l.), Anfu County, Jiangxi Province, China; Males SYS a 007269–7271, and females SYS a007272–7273, collected by Zhi-Tong Lyu, Yu-Long Li, and Yao Li on



**Figure 12.** Morphological features of the adult male holotype SYS a006492 of *Nidirana xiangica* sp. nov. in preservative. (A) dorsal view; (B) ventral view; (C) lateral view; (D) left hand; (E) right foot.

21 June 2018 from Mt Yangming (26.1155°N, 111.9591°E; ca 1150 m a.s.l.), Shuangpai County, Hunan Province, China.

**Etymology.** The specific name *xiangica* is an adjective derived from Xiang (湘), referring to Xiangjiang River (湘江), the major drainage basin within the distribution of the new species.

**Differential diagnosis.** *Nidirana xiangica* sp. nov. is distinguished from its congeners by the following combination of the morphological characteristics: (1) body large and elongated, with SVL 56.3–62.3 ( $58.0 \pm 2.2$ ,  $N = 6$ ) mm in adult males, and SVL 53.5–62.6 ( $58.3 \pm 4.0$ ,  $N = 4$ ) mm in adult females; (2) disks of digits dilated, rounded; (3) lateroventral grooves present on all digits; (4) heels just meeting; (5) tibio-tarsal articulation reaching between eye to snout; (6) mid-dorsal stripe absent; (7) dorsal surface and flanks extremely rough with dense tubercles; (8) developed supernumerary tubercles below the base of each finger, palmar tubercles prominent and distinct; (9) supratympanic fold absent; (10) white horny spinules on the entirely dorsum, dorsolateral folds, flanks, dorsal limbs, loreal region, and temporal region including tympanum in males; (11) a pair of subgular vocal sacs present; (12) one single nuptial pad on the finger I, nuptial spinules invisible; (13) suprabrachial gland large, rough and well developed, distinctly prominent; (14) calling: 2–3 notes containing a specific first note.



**Figure 13.** (A) adult female paratype SYS a006491 of *Nidirana xiangica* sp. nov.; (B) adult female paratype SYS a007273; (C) adult male paratype SYS a006493; (D) adult male paratype SYS a007269.

**Comparison.** Morphologically, *Nidirana xiangica* sp. nov. is unique when compared with all known congeners by the combination of the following characteristics: (1) large body size, SVL 56.3–62.3 mm in males and 53.5–62.6 mm in females vs. < 53.0 mm in males or females in *N. nankunensis*, *N. okinavana*, *N. daunchina*, *N. yaoica*, *N. chapaensis*, and *N. hainanensis*; (2) relative finger lengths  $II < I < IV < III$  vs.  $II < I = IV < III$  in *N. chapaensis*; vs.  $II < IV < I < III$  in *N. leishanensis*; (3) presence of lateroventral groove on every digit vs. absent on fingers and toes in *N. pleuraden*; vs. absent or barely visible on fingers in *N. daunchina*; vs. absent on finger I in *N. guangdongensis*, *N. mangveni*, *N. adenopleura*, *N. nankunensis*, *N. okinavana*, *N. chapaensis*, and *N. lini*; (4) tibio-tarsal articulation reaches between eye to snout vs. beyond the snout tip in *N. lini*; (5) heels just meeting vs. overlapping in *N. guangdongensis*, *N. mangveni*, *N. adenopleura*, *N. nankunensis*, *N. yaoica*, *N. leishanensis*, *N. okinavana* and *N. lini*; (6) white horny spinules on the entirely dorsum, flanks, loreal region, and temporal region including tympanum in males vs. absent on dorsum and flanks or few above vent in *N. nankunensis*, *N. okinavana*, *N. daunchina*, *N. yaoica*, *N. chapaensis*, *N. leishanensis* and *N. hainanensis*; vs. present on dorsum while absent on flanks in *N. mangveni*, *N. adenopleura*, *N. lini* and *N. pleuraden*; vs. present on dorsum and flanks while absent on temporal regions in *N. guangdongensis*; (7) the presence of a single nuptial pad on finger I vs. absent in *N. hainanensis*; vs. divided into two parts in *N. chapaensis*; vs. two

nuptial pads on fingers I and II respectively; (8) the presence of a pair of subgular vocal sacs vs. absent in *N. okinavana*.

**Description of holotype.** SYS a006492 (Figs 11, 12), adult male. Body large and elongated, SVL 56.3 mm; head slightly longer than wide (HDW/HDL 0.99), flat above; snout rounded in dorsal and lateral views, slightly protruding beyond lower jaw, longer than horizontal diameter of eye (SNT/ED 1.27); canthus rostralis distinct; loreal region concave, bearing horny spinules; nostril round, directed laterally, closer to the snout than to the eye; a longitudinal swollen mandibular ridge extending from below nostril through lower edges of eye and tympanum to above insertion of arm, forming a maxillary gland and shoulder gland; supratympanic fold absent; interorbital space flat, narrower than internasal distance (IND/IOD 1.27); pupil elliptical, horizontal; temporal region including tympanum with horny spinules, tympanum distinct, round, TD/ED 0.81, and close to eye, TED/TD 0.29; pineal ocellus distinct; vomerine ridge present, bearing small teeth; tongue large, cordiform, notched behind; a pair of subgular vocal sacs present.

Forelimbs moderately robust, lower arm 0.20 of SVL and hand 0.26 of SVL; fingers thin, relative finger lengths  $II < I < IV < III$ ; tip of each finger slightly dilated, forming rounded disks; lateroventral grooves on all fingers, not meeting at the tip of disks; fingers free of webbing; presence of distinct lateral fringes on inner and outer sides of fingers II, III, and IV, and on outer side of finger I; subarticular tubercles prominent and rounded; developed supernumerary tubercles below the base of each finger; three elliptic, large, prominent and very distinct palmar tubercles; a single nuptial pad on the dorsal surface of first finger, nuptial spinules invisible.

Hindlimbs relatively robust, tibia 0.50 of SVL and foot 0.74 of SVL; heels just meeting when hindlimbs flexed at right angles to axis of body; tibio-tarsal articulation reaching the loreal region when hindlimb is stretched along the side of the body; toes relatively long and thin, relative lengths  $I < II < V < III < IV$ ; tip of each toe slightly dilated with remarkable elongated ventral callous pad, forming long and pointed disk; well-developed lateroventral grooves on toes, not meeting at the tip of disks; webbing moderate, webbing formula:  $I 1\frac{1}{2} - 2 II 1\frac{1}{3} - 2\frac{1}{3} III 1\frac{2}{3} - 3 IV 3\frac{1}{3} - 1\frac{2}{3} V$ ; presence of lateral fringes on inner and outer sides of each toes, forming distinct dermal flap on the lateral edges of toes I and V; subarticular tubercles rounded, prominent; inner metatarsal tubercle elliptic, length triple the width; outer metatarsal tubercle indistinct, small and rounded; tarsal folds and tarsal tubercle absent.

Dorsal surface very rough with dese tubercles and dense horny spinules; developed dorsolateral fold with sparse horny spinules from posterior margin of upper eyelid to above groin but intermittent posteriorly; flank very rough with sparse warts, dense tubercles and dense horny spinules; a large and rough suprabrachial gland behind base of forelimb, distinctly prominent; dorsal surface of forelimb rough with dense horny spinules, two weak longitudinal ridges on upper arms and slightly extending to lower arm; the dorsal surfaces of thigh and tibia rough with dese tubercles and dense horny spinules, forming several longitudinal ridges. Ventral surface of

throat, body, and limbs smooth; large flattened tubercles densely arranged on the rear of thigh and around vent.

**Coloration of holotype.** In life (Fig. 11), dorsal surface greenish brown; horny spinules on the skin white; pineal ocellus yellowish; absence of mid-dorsal stripe; dorsolateral fold greenish brown; upper flank greenish brown, warts on flank yellowish; lower flank yellowish white with black stripe; suprabrachial gland yellowish white with black stripe. Dorsal limbs brown; two greenish crossbars on the thigh, two on the tibia and three on the tarsus. Loreal and temporal regions greenish brown, tympanum light brown; upper  $\frac{1}{3}$  iris brownish white and lower  $\frac{2}{3}$  iris reddish brown; maxillary gland and shoulder gland white. Throat and anterior chest dark purplish brown; ventral surface of body and limbs creamy white; rear thigh tinged with pink; ventral hand white with large brown patches; ventral foot purplish brown.

In preservative (Fig. 12), surface of dorsum and dorsal limbs changed as dark brown; white spinules significantly distinct; crossbars on limbs clearer; ventral surface faded, throat and anterior chest dark grey.

**Variations.** Measurements of type series are given in Table 8. All specimens were similar in morphology. Females ( $58.3 \pm 4.0$  mm,  $N = 4$ ) (Fig. 13A, B) are not significantly larger than males ( $58.0 \pm 2.2$  mm,  $N = 6$ ), but relatively smooth than males, not bearing white horny spinules on the dorsum, dorsolateral folds, flanks, and temporal region. Pineal ocellus invisible in SYS a006493 (Fig. 13C); dorsal surface reddish brown in SYS a006491 and greenish in SYS a007269 (Fig. 13D); numerous black spots on dorsum and flanks in SYS a007273; lateroventral grooves poorly developed on fingers I and II in SYS a002591.

**Distribution and ecology.** Currently, *Nidirana xiangica* sp. nov. is known from Mt Dawei and Mt Yangming of Hunan, Mt Wugong of western Jiangxi, and Mt Du-

**Table 8.** Measurements (in mm) of the type series of *Nidirana xiangica* sp. nov. An asterisk denotes the holotype.

	SYS a006492 *	SYS a006493 /CIB 107276	SYS a002591	SYS a007269	SYS a007270	SYS a007271	SYS a006491	SYS a002590	SYS a007272	SYS a007273
Sex	Male	Male	Male	Male	Male	Male	Female	Female	Female	Female
SVL	56.3	62.3	57.1	57.7	56.5	57.9	53.5	56.8	62.6	60.2
HDL	20.0	23.1	19.7	21.2	22.0	22.4	19.6	21.2	24.1	22.5
HDW	19.8	22.0	19.4	20.0	19.0	20.7	18.7	20.7	22.2	18.9
SNT	8.0	9.5	8.6	8.7	8.7	8.1	7.8	8.4	9.0	8.4
IND	6.6	7.1	6.5	6.5	6.4	6.7	6.3	6.4	7.0	6.8
IOD	5.2	5.5	5.3	5.1	5.0	5.0	5.0	5.0	5.8	4.9
ED	6.3	6.8	5.9	6.3	6.0	6.6	5.9	6.4	6.2	6.0
TD	5.1	5.6	5.2	5.2	5.7	5.7	4.9	5.0	5.3	4.9
TED	1.5	1.7	1.6	1.5	1.5	1.5	1.5	1.7	1.7	1.7
HND	14.6	15.2	15.2	14.8	14.2	15.0	14.5	15.4	15.8	15.3
RAD	11.3	12.0	11.3	11.1	11.3	11.1	11.1	12.0	12.0	11.1
FTL	41.4	45.2	47.0	43.5	43.8	45.6	42.0	46.6	48.5	45.5
TIB	28.3	31.5	32.0	29.7	30.3	30.2	30.0	32.1	32.0	30.8

pangling of northeastern Guangxi, indicating its potential distribution area is in the Xiangjiang River Basin. The frog inhabits natural or artificial ponds and paddy fields. This species has no behavior of nest construction, and the adult males call at the water surface from May to August. The tadpoles of this species remain unknown.

**Vocalization.** The advertisement call ( $N = 57$ ) of *Nidirana xiangica* sp. nov. contains 2–3 notes containing a specific first note. The two-note call has a duration of 331.9–427.0 ( $374.6 \pm 23.5$ ,  $N = 19$ ) ms; the three-note call has a duration of 542.7–624.8 ( $569.2 \pm 20.6$ ,  $N = 38$ ) ms. The first notes last 148.0–233.0 ( $170.4 \pm 14.5$ ,  $N = 57$ ) ms with the rise time 89.8–149.1 ( $126.2 \pm 17.5$ ,  $N = 57$ ) ms; the non-first notes last 60.1–128.0 ( $74.6 \pm 11.8$ ,  $N = 95$ ) ms with the rise time 2.2–43.0 ( $27.8 \pm 10.2$ ,  $N = 95$ ), and the intervals last 85.0–195.6 ( $125.8 \pm 17.8$ ,  $N = 95$ ) ms.

## Discussion

In morphology, most anuran species seem slightly similar to each other, and within several particular species, the coloration patterns are variable among individuals. These interspecific similarities and intraspecific variabilities have caused numerous misidentifications and synonymies, and calls for comprehensive approaches in the taxonomic research on anuran frogs. For instance, the species *Nidirana guangdongensis* sp. nov. and *N. mangveni* sp. nov. overlap with each other in the morphometric comparisons, while detailed morphological comparison, phylogenetic relationships, and bioacoustics analysis reveal their differences. The species *N. xiangica* sp. nov. is significantly different from *N. adenopleura* s. s. in morphology, phylogeny, and bioacoustics, but it was previously misidentified as *N. adenopleura* possibly to deficiencies in earlier research.

The species *Nidirana adenopleura* was originally described based on several specimens from Fuhacho Village (= Maobu or Wucheng, Nantou County), central Taiwan (Boulenger 1909; Jang-Liaw and Chou 2015), and was subsequently recorded over a wide area from southern China to northern Indochina (Chan-ard et al. 1999; Fei et al. 2009, 2012). Fei et al. (2007) described the reported population of *N. adenopleura* from Mt Diaoluo, Hainan as the new species *N. hainanensis*. Chuaynkern et al. (2010) re-allocated the specimens previously identified as *N. adenopleura* from Thailand to *N. lini*. Lyu et al. (2019) recognized the population from Mt Dayao, Guangxi as the new species *N. yaoica*. These taxonomic works have indicated that the current records of *N. adenopleura* represent a species complex. In the present study, based on a comprehensive molecular, morphological, and bioacoustics analysis, the recorded populations of *N. adenopleura* from Nanling Mountains and southern Luoxiao Mountains (southern lineage), northern Zhejiang (northern lineage), and Xiangjiang River Basin (western lineage), are revealed as the new species *N. guangdongensis* sp. nov., *N. mangveni* sp. nov., and *N. xiangica* sp. nov. Currently, the recognized distribution area of the true *N. adenopleura* covers the Taiwan Island, northern Fujian, southern Zhejiang and central Jiangxi, and other reported populations beyond these areas need further study.

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## Appendix

### Specimens examined

- Nidirana adenopleura* (29): **China: Fujian:** Yanping District: SYS a005911–5916 (topotypes of the junior synonym *N. caldwelli* (Schmidt, 1925)); Mt Wuyi: SYS a005939–5943; Jiangshi Nature Reserve: SYS a004112, 4132; Mt Yashu: SYS a005890–5891, 5901–5902; **Jiangxi:** Tongboshan Nature Reserve: SYS a001663–1665, 1667, 1698; Yangjifeng Nature Reserve: SYS a0000317, 0334; Jinggangshan Nature Reserve: SYS a004025–4027; **Zhejiang:** Jingning County: Dongkeng Town: SYS a002725–2726.
- Nidirana daunchina* (5): **China: Sichuan:** Mt Emei: SYS a004594–4595 (topotypes); Hejiang County: Zihuai Town: SYS a004930–4932.
- Nidirana hainanensis* (4): **China: Hainan:** Mt Diaoluo: SYS a003741, 7669–7671 (topotypes).
- Nidirana leishanensis* (3): **China: Guizhou:** Mt Leigong: SYS a007908 (topotypes); Mt Fanjing: SYS a007195–7196.
- Nidirana lini* (4): **China: Yunnan:** Jiangcheng County: Hongjiang Town: SYS a003967–3970 (topotypes).
- Nidirana nankunensis* (12): **China: Guangdong:** Mt Nankun: SYS a003615, 3617–3620, 4019, 4905–4907, 5717–5719 (holotype and paratypes series).
- Nidirana pleuraden* (4): **China: Yunnan:** Mt Gaoligong: SYS a003775–3778.
- Nidirana yaoica* (13): **China: Guangxi:** Mt Dayao: SYS a007009, 7011–7014, 7020–7022, NHMG 1503043–47 (holotype and paratypes series).

